PRECLINICAL SAFETY EVALUATION OF HERBO MARINE FORMULATION-PALAGARAI CHUNNAM

The dissertation Submitted by

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"**Preclinical safety evaluation of Herbo marine formulation-**Palagarai chunnam**" is a bonafide and genuine research work carried out by me under the guidance of **Dr.V.MANJARI, M.D(S)., Guide, Department of Nanju Noolum Maruthuva Neethi Noolum**, National Institute of Siddha, Chennai -47, and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or another similar title.

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BONAFIDE CERTIFICATE

Certified that I have gone through the dissertation submitted by **Dr.S.Balamurugan**, (**Reg .No: 321516201**) a student of final year M.D(S), Branch-VI, **Department of Nanju Noolum Maruthuva Neethi Noolum**, National Institute of Siddha, Tambaram Sanatorium, Chennai-47, and the dissertation work has been carried out by the individual only. This dissertation does not represent or reproduce the dissertation submitted and approved earlier.

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INTRODUCTION

1. INTRODUCTION

The siddha system has its entire literature in Tamil language. This system of medicine was developed by the 18 ancient saints who were known by their name siddhars whose life goal was to attain the salvation. The siddha system of medicine uses a fascinating combination of herbs, minerals and metals and to promote good health and longevity[1]. According to siddha system, the universe originally consisted of atoms which contributed to the basic elements,viz., Earth, Water ,Fire ,Air and the Sky which correspond to the organs of the human body and they were the fundamentals of all the corporeal things in the world. The history of siddha medicine is to present a faithful, clear , and vivid picture of this system in all its manifestations and ramifications with all its inherent problems and relevancy to the present age from its very beginning down the ages , as an integral component of the patterns of culture through which this system passed in different ages and in different areas, so that , this age can get to know of its uniqueness in several aspects

Its very clear and evident from long history of usage of herbomineral and metallic preparations in Ayurveda and Siddha medical system that properly processed herbomineral preparation can contribute significantly to the health care of the society. To understand the science involved in the purification processes a simple method was selected and studied. The common techniques used for the purification of a particular compound are based on its nature and also on the nature of the impurities present in it.

Siddha medical system that properly processed herbomineral formulation can contribute significantly to the health care of the society [2].Siddha medicines are formulated based on the concept of "*Panjabhootha* theory" and "*Arusuvai*kal *"* and on the basis of three humours (*vatham*, *pitham*, *kabham*). The prevalence of female infertility Number of married women aged 15-44 that are infertile is 1.0 million. Number of women aged 15-44 who have ever used infertility services: 6.9 million. Globally, anaemia affects 1.62 billion people (95% CI: 1.50–1.74 billion), which corresponds to 24.8% of the population (95% CI: 22.9–26.7%). The highest prevalence is in preschool-age children (47.4%, 95% CI: 45.7–49.1), and the lowest prevalence is in men (12.7%, 95% CI: 8.6–16.9%)[(3)].

The prevalence of dysmenorrhea in adolescent girls was found to be 79.67%. However, the population group with the greatest number of individuals affected is non-pregnant women (468.4 million, 95% CI: 446.2–490.6). Because of the increased prevalence there is an emergence need of an effective drug for the management of female infertility, Dysmenorrhea, anaemia and other chronic diseases[(4)].

Palagarai chunnam is one of the traditional Siddha formulation which is indicated as a best drug for Female Infertility, Dysmenorrhea, Anemia, Dropsy in Siddha text ***Siddha maruthuva nool thirattu - Anubhava Siddha Vaithiya Muraigal*****.** Scientific validation of this formulation *Palagarai chunnam* have to be studied and the safety of the drug have to be ensured.

2. AIM AND OBJECTIVES

AIM

To evaluate the safety profile of (Acute and 28days Repeated dose oral Toxicity study) **"Palagarai chunnam"** in Wistar albino rats.

OBJECTIVE

- Collect the literature review of ingredients of *Palagarai chunnam*
- Purification and preparation of the Medicine as per literature
- To Analyses the physical and chemical properties of ***"Palagarai chunnam".***
- Acute oral toxicity study & 28days repeated oral toxicity study of ***Palagarai chunnam*** as per OECD Guideline 423 & 407.
- Evaluation of safety of the study drug

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1.பலகறை

வேறுபெயர் : கவடி ,சோகி, வராடி

சுவை : கைப்பு

செய்கை : தாதுவெப்பகற்றி
கோழையகற்றி
வெளிபிரயோகத்தில் தடிப்புண்டாக்கி

நிறம் : வெள்ளை, மஞ்சள், சிவப்பு
வெண்ணிற பலகறையே சிறந்தது

அளவு : புளியின் வித்து முதல் வாதுமை கொட்டை பருமன்

பொது குணம்:

"மந்ததந்தா கங்கிரகணி மாவிடச் சுரங்கண்ணோய்
தொந்தம் பரிநாமச் சூலைகய – மிந்த
வுலகறையைக் காலொடிவை யோடு நரைத்த
பலகறையை காணினியம் பார்".[5]

பலகறை பற்பம்:

ஒருபலம் சுத்தி செய்த பலகறையை எடுத்து கீழ் உள்ள பட்டியில் குறித்த முறைப்படி அரைத்து உலர்த்தி புடம் இடவும். ஓவ்வொரு நாளும் குறித்த புதிய சாற்றை உபயோகிக்கவும். வில்லையை உலர்த்த சூரிய ஒளியில் வைப்பது போல இரவில் பனியிலும் வைத்தல் வேண்டும்.

சாற்றின் பெயர்	சாற்றின் அளவு பலம்	அரைக்கும் நாள்	வில்லை உலர்த்தும் நாள்	கவசம் உலர்த்தும் நாள்	புடம் வரட்டி
சுரபுன்னை சமூலம்	4	6	5	1	36
சித்திரமூல சமூலம்	4	5	4	1	30
கல்லால் சமூலம்	4	4	3	1	24
காட்டுமல்லிகை சமூலம்	3	3	2	1	18
நீலோற்பல சமூலம்	2	2	1	1	12
சந்தன குழம்பு தெளிநீர்	1	1	1	1	6

அளவு:

கடலையில் 1:5 - உத்தமம்

கடலையில் 2:5 - மத்திமம்

கடலையில் 3:5 - அதமம்

கடலையில் 4:5 - அதமாதம்

முழுகடலை - ஆனந்தம்

தீரும் நோய்கள்:

கப நோய் - அனுபானம் -கள்

பித்த நோய் - அனுபானம்-வெள்ளை சர்க்கரை

வாத நோய் - அனுபானம் -மகிழம்பூரசம்

பலகறை பற்ப மகிமை:

இப்பற்பம் உடலிலிருந்து நீங்கிய வன்மையை திரும்ப கொடுத்து மகிழ்வை உண்டு பண்ணும்

பலகறை செந்தூரம்:

ஒரு பலம் சுத்தி செய்த பலகறைப் பொடிக்கு கீழ்க்கண்ட பட்டியலில் கொடுக்கப்பட்டுள்ள முறைப்படி சாறுகளை உபயோகிக்கவும்

1. மாம்பட்டை சமூலச்சாறு
2. ஆனைவணங்கி சாறு
3. விடத்தேரிச்சாறு
4. செருப்படைச்சாறு

இவைகளை பற்பத்திற்கு கூறிய அளவின் படி விட்டரைத்து வில்லை செய்துலர்த்திக் கவசித்து புடமிட்டெடுக்க செந்தூரமாம்.

அளவு: 2.5 (325மி.கி) முதல் 5 குன்றி (650 மி.கி)

தீரும் நோய்கள்: நீரெரிச்சல்

பிரயோகம்:

இதனை புண்ணுண்டாக்கி செய்கைக்காக உபயோகிப்பதுண்டு.

TREATMENT OF ANIMAL BITES WITH PALAGARAI PARPAM:

Table no: 2 Therapeutic application of Palagarai Parpam as an anti - dote:

S.no	Name of the animal	Adjuvant
1	**Dog**	
	i)Natural rabies	Juice of Curculigo orchoides (nilapanai) and cow gram gruel.
	ii)Induced rabies	Juice of banyan leaves and horsegram gruel
2	**Fox**	
	i)Natural rabies	Bamboo juice and dhal gruel
	ii)Induced rabies	Phyllanthus amarus (keezhanelli) and greengram gruel
3	**Cow**	
	i)Natural rabies	Betel leaves and little millet
	ii)Induced rabies	Achyranthus aspera (naayurivi) and kodo
4	**Buffalo**	
	i)Natural rabies	Nut grass and Italian millet
	ii)Induced rabies	Prosopis spicigera (vanni) juice with grass seeds gruel
5	**Pig**	
	i)Natural rabies	Juice of bitter wild snake gourd and wheat gruel
	ii)Induced rabies	Juice of bitter gourd and gruel of bamboo sago

6	**Human** i)Natural rabies ii)Induced rabies	Juice of mollugo lotoides (cheruppadai) and Creeper juice of jackal green gram and lablab seed gruel

USAGE OF PALAGARAI PARPAM FOR VARIOUS TYPES OF WOUNDS AS OINTMENT [5]

1. Wounds caused by thorns and ox-horn -Apply with Parrot's egg yolk

2. Wounds caused by palm leaf stalk -Apply with Hen's egg yolk

3. Wounds caused due to knife, spear, etc -Apply with pigeon's egg yolk

4. Wounds caused by Stonning and bruise caused by falling -Apply with Frog's egg yolk

5. Wounds caused due to ancient weapon (gadhai), Stick and pestle -Apply with Crow's egg yolk

6. Wounds caused by striking with hands and Pinching by nail - Apply with nightingale's egg yolk

7. Chronic wounds caused by various other means. -Apply with Tortoise's egg yolk

பலகறை சேரும் மருந்துகள்:

1.நயனரோகத்திற்கு மாத்திரை

அளவு : 1 மாத்திரை

முறை : முலைப்பாலில் இழைத்து கண்ணிலிட வேண்டும்

தீரும் நோய் : 20 வருடங்கள் சென்ற பூவும் மாறும்.[6]

2.நயன வியாதிக்கு மாத்திரை

அளவு : 1 மாத்திரை

முறை : பிள்ளைப் பாலில் இழைத்து கண்ணிலிட வேண்டும்

தீரும் நோய் : விழிநோய்கள் பலவும் தீரும்.[6]

3. ரத்தினாதி மாத்திரை

அளவு : கடுகளவு

முறை : தாய்ப்பாலில் இழைத்து இரு விழிகளிலிட வேண்டும்

தீரும் நோய்கள் : திமிரம், அமரம், விழிகாசவகை[6]

4. சிரோஸ்தான சிட்சிகாபரிசனம்

பலகறை : 10 பலம்

ஆறுமாத பழங்காடி : நாழி (1.3 லி)

நிம்ப பழச்சாறு : 6 பலம்

இவற்றை கலந்து வைக்க வேண்டும்

முறை : நசியம் கலிக்கம்

செய்து பின் 122 குடம் நீர் ஊற்ற வேண்டும்

தீரும் நோய்கள் : வாதம், பித்தம், பைத்தியம் குணமாகும்[7]

5 .காது சீழ் பொங்குதல்:

ஒரு பலகறையை அடுப்பில் நன்றாய் சுட்டு இடித்து தூளாக்கி ஒரு சிட்டிகை காதில் போட்டு எலுமிச்சம்பழ ரசம் கொஞ்சம் பிழியவும்[8].

6. விஷ்ணு சக்கர மாத்திரை

அளவு : 1 குன்றி

அனுபானம் : திரிகடுகு தூள், இஞ்சி நீர், தேன்

தீரும் நோய்கள் : பக்கவாதம், விக்கல், சோபை, 13 சன்னி, ஏப்பம், மூர்ச்சை, வாயு[9]

7. கார சூடசத்து பற்பம்

அளவு : 1- 1.5 பணவெடை

அனுபானம் : இளநீர், வெங்காய சாறு, பழரசம், தேன்

தீரும் நோய்கள் : நீரடைப்பு, கல்லடைப்பு, சதையடைப்பு, நீர்க்கட்டு

பத்தியம் : காரசாரமற்ற அன்னம் கொள்ள வேண்டும்[10]

1.1. PALAGARAI - Cypraea moneta

Zoological classification [11]:

Kingdom : Animalia

Phylum : Mollusca
Order : Littorinimorpha
Suborder : Cypraeoidea
Family : Cypraeidae
Genus : Cypraea
Species : moneta

Vernacular names [12] :

English : Porcelaneous shells, Marine shells, Money cowri, Cowri

Sanskrit : Varatika, Varataka

Arab : Sadaf

Persian : Khar – Mahra

Hindi : Cowri, Sipi

Bengali : Beya

Gujarati : Codi

Cannada : Kavdi

Telugu : Gavalu

Singalese : Pingo

Tamil : Palagarai

General description

Palagarai (Cypraea moneta) is known as the “Jewels of the sea”. Cyprea moneta is a very common species which is found widely in Indo-Pacific tropical waters. It is present in numerous regions, including East and South Africa, Madagascar, the Red Sea and the Persian Gulf, Maldives.

Cowries have a dome shaped and almost hemispherical shell, shell, size varies from 8mm to 150mm, ovate to pyriform, highly enameled and have a smooth or occasionally pustulose surface. Aperture moderately narrow, and the labial and columellar lips are denticulate; Fossula may smooth or ribbed, extremities truncate or produced; Base of the shell convex or flat. Spires absent in the adult, Juvenile shell is strikingly different from that of an adult. A spiral shell is present in the early stage but on maturity the outer lip turns in, thickness and teeth develop on it and the inner lip(columella). The shell then turns ovate pyriform, domed, globular or hemispherical[12].

There is a pair of long, filiform cephalic tentacles bearing eyes at their outer bases .Foot is broad and extensible. Mantle cavity contains an arched ctenidium with several lamellae, a triradiate osphradium and a large hypo-branchial gland. Radula is long and taenioglossate(2-1-1-1-2)..Sexes separate. Female lays eggs on coral substrate or in crevices of the reef. Egg mass is laid in either a circular or oblong shape and consist of layers of capsule in a cluster. Each egg mass with 100 to 300 eggs capsules. Egg capsules are various in colour like white, yellow, mauve or purple, orange, pink and each capsule with 200 to 500 eggs [13.]

“cowries” are inhabitant of coral reefs, and seek refuge from the sea under loose coral rocks, and hide in reef crevices and at the base of soft coral. Lives in warm waters of oceans.

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Worldwide dispersal of palagarai

Cowries (Cypraea moneta) are represented by about 200 species in all but maximum numbers of about 140 species occur in the indo pacific region. Over 50 species are known from Indian seas. Greatest diversity seen the reef ward edge and surf beaten zones in coral reef eco system of Andaman and Nicobar Islands, Gulf of Mannar, gulf of kanchchh and Lakshadweep.

Majority of the species inhabits shallow water, but a few may extend into the reef front that remains unexposed even during spring tide. The family is representing single genus, which is divided into more than 53 subgenera, some of which are elevated to a generic status by many authors [13].

As our currency was used for a good 4,000 years, from the end of the 3rd century BC to the second half of the 20th century AD. The porcelain-like shell of the cowry circulated around the globe longer than any other currency in the history of money.

The oldest written evidence of cowries being used as money was found in China. Evidence of the immense importance of the cowry in the history of Chinese coinage is the fact that our modern day symbol for money has its origin in the stylized image of a cowry.

The shell of the cowry, also known as “porcelains” in Old Italian, was highly coveted. Her Latin name *Cypraea moneta* indicates that the shell served as money for centuries. This cowry string is originally from Africa.

Cowries were exported to Africa since the 14th century, first by Arabic, then by English and even by German merchants. Today, the coins and banknotes of several states still refer to this ancient currency as this banknote from the Maldives shows.

Varieties:

Three varieties of cowries white, red and yellow are used in medicine. Ancient Alchemists preferred yellow colour. Cypraea moneta Linnaeus, 1758, is an abundant and easily recognized gastropod throughout the Indo-West pacific. A number of names distinguishing subspecies and races have been introduced for the money cowry, and it is generally recognized as a highly variable species. Different species of cowries are dependent on the

Genetic factor

Pigmentation

Disease

Injury

Presence or absence of aluminum and other compounds

The acidity of the soil and water

Temperature of water.

Primarily genetic abnormalities, injury, disease and environmental factors can for instance lead to albinism, while certain diseases produce unusually large and heavy shells with a calloused, mottled appearance[14].

Palagarai in astrology

A 'Kavadi' in Tamil stands for a sea shell. Using these, the position of an individual and the cause of his visit for consultation is found and the solution of his problems are suggested. For this, 108 kavadis are used and they are rotated a number of times, the blessings of Guru are invoked.

A portion of the kavadis are separated and counted to find out the ruling planet at that time. The results of the prasna horoscope (A horoscope formulated at the time of arrival of the persons) are compared with the results of the kavadi prasnam, and the predictions are pronounced[15].

RECENT RESEARCHES ABOUT PALAGARAI:

1. Cardiac activity and Analgesic activity

The presence of cardenolides contributes to the role of the deadly venoms of some cowrie shells which are used today to help victims of strokes, heart diseases and produce revolutionary new drug for chronic pain control[16].

2. Anti-inflammatory activity

Powdered Pearls from shells are used as topical Eye medicine. It has been scientifically proved to has some anti-inflammatory effect on conjunctivitis where the surface of the eye become red and sore[17].

3. Antipyretic, anti - inflammatory, anti - microbial activity

In the study, the efficacy of drug prepared from the shell of mollusk Cypraea moneta to reduce fever and heal wounds in albino rats as well as to inhibit microbial activity in vitro. This drug efficiently reduced the body temperature of rats that was made hyperthermia by yeast injection. Similarly, the wound healing process ending with the production of scar indicated that tissue regeneration was completed in drug administered rats. Sometimes pathogenic microbes can enter through the wound and produce pus. In this experiment, treated rats did not produce pus on the contrary to control rats. Thus Cypraea moneta is found to be effective in anti-pyretic, anti-inflammatory, anti-microbial in experimentally induced albino rats.[18]

2.CITRUS LEMON

BOTANICAL CLASSIFICATION[13]

- Kingdom – Plantae, Angiosperms, Eudicots, Rosids
- Order – Sapindales
- Family – Rutaceae
- Genus – Citrus
- Species – C. limon
- Binomial name - *Citrus × limon*

DIFFERENT NAME IN LEMON[14]

- English: Lemon, Lime
- Gujarat: Limbu, Motu limbu
- Hindi: Nimbu
- Kannadam: Nimbe
- Malayalam: Cherunakaram
- Tamil: Elumicahi
- Telungu: Jambhira nimma

Description

Much branched thorny shrub leaves ovate, Petiole slightly winged. Flowers are white, axillary, solitary or clustered. Fruits oblong or ovoid, Bright yellow with terminal nipple, pericarp thick and seeds many.

Distribution

Throughout India, Cultivated in plains and hills in area upto 1,200m elevation.

Habit – cultivatd in India, the terminal in the C.P., Kumaon and Northern India.

Varieties- Two kind of limes are found in the Indian market. The lemon though belonging to the same stock.

Parts Used- Rind of the ripe fruits and expressed juice of the ripe fruits.

Constituents- A pale yellow volatile oil derived on either by distillation or by simple expression from the fresh outer part of the pericarp or finely grated rind of the fruit. Lemon is richer in juice and citric acid than lime. The average amount of citric acid available from 100 c.c.pf lemon juice is 3-7 percent.

Action:

Stomachic and carminative

Oil:

It is bitter, aromatic, stomachic and carminative n doses of from 2 to 4 drops but is rarely employed in this form.

Juice:

The expressed strained juice of the ripe fruit is a valuable antiscorbutic and refrigerant , primarily anti alkaline and secondarily antacid .

Bark:

It is used as febrifuge and seeds as a vermifuge. Pulp is exceedingly acid

Medicinal Uses of Lemon Juice

- Lemon Juice and gun powder is applied topically for scabies.
- Juice of the baked lemon is an excellent remedy for cough when mixed with an equal quantity of sugar or honey and taken in tea spoonful doses.
- Fresh lemon juice is recommended to be taken in the evening for the relief of dyspepsia with vomiting and bilious headaches.
- Preserved with sugar or honey lemons are recommended for sore throat and are considered to act as detergent they are administered before purgatives to prepare the body for them and afterwards to check excessive action.
- Lemon plays an important part in perfumery also. The quality of Indian lemon peel is almost equal to the Sicilian variety and it has been estimated that if extraction of lemon oil is attempted from the Indian lemon Peel, It will not be a failure commercially[15].

The fruits in the form of pickles is useful in hypertrophy of spleen.. Lemon peel is stomachic and carminative. Oil of lemon is stimulant and rubifacient when applied externally. Lemon juice is one of the best remedies for scurvy and serves as a refrigerant in febrile and inflammatory affections, acute rheumatism, dysentery and diarrhoea . The fruit is digestive carminative, stomachic, laxative, anthelmintic, stimulant, antiseptic and is useful in flatulence, dyspepsia, constipation, colic and helminathiasis [16].

Nutritional value per 100 g of Lemon Juice [17]

- Energy - 129 kcal
- Carbohydrates - 10.9 g
- Protien - 1.5 g
- Fiber - 1.3 g
- Calcium - 90 g
- Phosphors - 20mg
- Iron - 0.3mg
- Thymine - 0.02mg
- Riboflavin - 0.03,mg
- Vitamin C - 64 mg
- Energy - 59 Kcal

RECENT RESEARCHES ABOUT LEMON JUICE:

1. Diuretic and Anti -Hypertension Activity

Lemon juice is of value in Hypertension and Urinary diseases if used in the form of reconstituted Lemon drink (from Powder packet. Traditionally lemon juice has a vast number of uses including its anti-oxidant properties, anxiolytic, antidepressant effect as well as diuretic potential [18].

2. Health and Medicinal properties of lemon

Vitamin C present in the Lemon Juice. So it cures Scurvy**.** Lime juice and its oil are very beneficial for skin when consumed orally or applied externally. Lime juice has an irresistible scent which waters the mouth and thus aids primary digestion. Primarily, the ample of acids present in lime helps clear the excretory system by washing and cleaning off the tracts, just like some acids are used to clean floor and toilets. An overdose of lime juice with salt also acts as an excellent purgative without any side effects, thereby giving relief in constipation[19].

3. Antibacterial Activity of Fruits against Escherichia coli

The Lemon Juice contains Antibacterial Activity against E.coli. ƒ More organisms can undoubtedly be analyzed for this antibacterial activity. Numerous fruits are unquestionably utilized to prevent foodborne illness diseases [20].

4. Lemon Polyphenols Suppress Diet-induced Obesity

Feeding with lemon polyphenols suppressed body weight gain and body fat accumulation by increasing peroxisomal β-oxidation through up-regulation of the mRNA level of ACO (acetyl CoA oxidase) in the liver and white adipose tissue, which was likely mediated via up-regulation of the mRNA levels of the peroxisome proliferator activated receptor-α (PPARα) [21].

3. EUPHORBIA NERIIFOLIA, Linn, E. lingularia. (Euphorbiaceae)

Other Names:

Tamil - Ilaikalli, Kalli,

English – Common milk hedge

Hindi – Pattonkisend, sij,

Malayalam – Ilaikalli, kalli

Telugu – Akujemudu

Urdu – Zakum

Marathi – Mingut

Bengali - Mansasij

Habitat: This leafless shrub is found in central india and cultivated in Bengal.

Parts used: juice and root.

Constituent's: Euphorbon, resin, gum, caoutchouc, malate of calcium etc.

Action: Juice is purgative and expectorant, locally rubefacient like that of E. antiquorum. Root is antispasmodic.

Uses

Milk juice exuded from injured fleshy cylilndrical stems is used by Vaidyas in medicine as drastic cathartic and to relieve earache. Cloves, long-peppers, chebulic myrobalans and *trivrit* root etc., are soaked in this juice for some months and then dried, and used as a drastic purgative in the enlargement of liver and spleen, syphilis, dropsy, general anasarca, leprosy etc[28].

For instance

Take cloves 4 ounces and soak them into on seer of the milk for 40 or 50 days, then rub the whole into a mortar, the weight of this highly perfumed mass will be 12 ounces, now mix well in this mass, 360 grains of Rasakarpur called ”corrosive sublimate” of this whole 180 pills are prepared.

Two of such pills are administered to a patient at bed time, coated with a little fresh cream, so that the pills may be swallowed carefully without touching teeth. From the early morning till 10 a.m cathartic action will continue with watery stools. The patient should be given lukewarm aqua-ani seed 2 to 4 ounces after every motion.

Bread with butter freely should be given as a diet. In 20 to 40 days suffering with any of above disease is cured, as has been seen in a number of such bad cases (Gupta). As expectorant, especially in asthma, it is given in doses of 5 drops, mixed with a little honey of syrup.

Dr. M.C. Koman tried it and found it very beneficial in asthma, He prepared a succus consisting of equal parts of the juice of this plant and simple syrup and administered it in doses of 10 to 20 minims three times a day in cases of asthma and found it to relieve fits completely[28].

The plant is bitter, pungent, laxative, carminative, alexipharmic, improves the appetite, useful in abdominal troubled, bronchitis, tumors, loss of consciousness, delirium, leucoderma, piles, inflammations, enlargements of the spleen, anemia, ulcers, fevers.

The milk is pungent, laxative, good for abdominal troubles, tumors.

Leucoderma

The leaves are heating carminative improve the appetite, good for tumors, pains, inflammations, abdominal swellings. (Ayurveda).

The therapeutic properties are the same as those of E. tirucalli (Unani).

The milky juice is used as a purgative and rubfacient. It enters into the composition of most of the drastic purgatives.

The juice is employed in earache; mixed with soot it is applied to the eye in ophthalamia.

The juice of the leaves is a popular cure for earache in the Philippine islands.

A success consisting of equal parts of the juice of this plant and simple syrups was prepared and administered in doses of 10 to 20 minims three times a day in cases of asthma, and was found to gibe relief to the fits of that disease. (Koman).

The root is usefull in the antidotal and symptomatic treatment of snake bite (Mhaskar and Caius) and scorpion sting (Caius and Mhaskar), and equally usefull as an external application[29]

RECENT RESEARCH ABOUT E. NERIIFOLIA

1. Pharmacological activities:

Various plant parts or whole E. nerifolia extract and its isolates have been reported scientifically using various in-vivo and in-vitro experimental methods for anesthetic, analgesic, anti-anxiety, anti-convulsant, anti-psychotic, anti-arthritis, anti-carcinogenic, anti-diabetic,anti-diarrhoeal, anti-inflammatory, anti-thrombotic, antimicrobial, antioxidant, antiulcer, cytotoxic, death-receptor expression enhancing, dermal irritation, diuretic, hemolytic ,immunomodulatory, radio protective, scorpion venom and wound healing properties[30].

2. Anti-Bacterial activity:

Antibacterial effect was found in the ethanol and chloroform extract of E.neriifolia when was tested against the organisms and it was believed to be due to the presence of tannins and flavonoids which have been shown to possess antibacterial properties[31].

3. Antiviral activity:

Among of 23 compounds were isolated from ethanolic extract of leaves, 3-β-friedelanol exhibited more potent anti-viral activity than the positive control, actinomycin D implying the importance of the friedelane skeleton as a potential scaffold for developing new anti-HCoV-229E drugs[32].

4. MATERIALS AND METHODS

4.1 PALGARAI CHUNNAM:

4.1.1. Ingredients of Palagarai chunnam:

1. Palagarai
2. Lemon
3. Ilaikalli

4.1.2. Procurement and Authentication of Raw drugs:

Palagarai were procured from authenticate source of Raw Drug shop at Chennai. Identification and authentication done at Zoological survey of India, Chennai. The Herbs, Citrus limon(Lemon) and Ilaikkalli (Euphorbia nerifolia Linn.) were identified and authenticated by Assistant Professor, Department of Medicinal Botany, National Institute of Siddha, Chennai-47.

4.2 PREPARATION OF PALAGARAI CHUNNAM:

4.2.1 Purification of palagarai:

.	1.Palagarai **(** Cypraea moneta)	- 100gm
	2.Elumichai Pazha Saaru (Citrus limon)	- 300ml

Take the above mentioned quantity of Palagarai kept immersed in juice of lemon up to 24 hours[4] . Then wash those *Palagarai* with pure water and then dried in sun light.

4.2.2 Method of preparation:

Take the above mentioned quantity of Palagarai **(** C*ypraea moneta*) kept immersed in juice of lemon upto 24 hours. Then wash those Palagarai by using water. Those purified *Palagarai* have to be kept inside the 200g of Grinded Ilaikalli *(Euphorbia nerrifolia* Linn..) Leaves and it is covered by 5 layers of mud sealed cloth and dried well. Then it will be subjected into *putam* by using 30 cow dung cakes[33]. After incineration remove the mud sealed cloth and collect the *chunnam*. Then it will be grind and have to be kept in air tight container.

Route of administration: Oral

Dose: 130 mg

Adjuvant: Cow’s Milk

1. Purification of Palagarai

2.Palagarai before and after purification

3. Ilaikkalli karkam

Griended ilaikalli

4. Palagarai kept inside the Ilaikkalli karkam

5. Palagarai contained Ilaikkalli sealed by Mud plaster

6. Final product –Palagarai chunnam

ANALYTICAL STUDIES OF PALAGARAI CHUNNAM

The test drug (Palagarai chunnam) was subjected to following analytical studies like physicochemical analysis, Biochemical Analysis and Quantitative analysis by using sophisticated instruments.

4.3 QUALITATIVE ANALYSIS

The test drug (Palagarai chunnam was studied by physicochemical parameters. This study was done at The Tamil Nadu Dr. M.G.R. Medical University No.69, Anna Salai, Guindy, and Chennai-600032.

4.3.1 PHYSIOCHEMICAL ANALYSIS OF –PALAGARAI CHUNNAM

1. **Loss On Drying:**

An accurately weighed 2g of ***Palagarai Chunnam*** formulation was taken in a tarred glass bottle. The crude drug was heated at 105^0C for 6 hours in an oven till a constant weight. Percentage moisture content of the sample was calculated with reference to the shade dried material.

2. **Determination of total ash:**

Weighed accurately 2g of ***Palagarai Chunnam*** formulation was added in crucible at a temperature 600^0C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

3. **Determination of acid insoluble ash:**

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffler furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.

4. **Determination of water soluble ash:**

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450^0C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

5. Determination of water soluble Extractive:

5gm of air dried drug, coarsely powered ***Palagarai Chunnam*** was macerated with 100ml of distilled water in a closed flask for twenty-four hours shaking frequently. Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100^0 C and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

6. Determination of alcohol soluble extractive:

2.5gm. of air dried drugs, coarsely powdered ***Palagarai Chunnam*** was macerated with 50 ml. alcohol in closed flask for 24 hrs. With frequent shaking it was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^0C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

4.3.2. BIOCHEMICAL ANALYSIS

The bio-chemical analysis of Palagarai chunnam as done at Biochemistry lab National Institute of Siddha, Chennai-47.

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
1.	**Appearance of the sample**	White in color	
2.	**Solubility:** A little of the sample is shaken well with distilled water	Sparingly soluble	Presence of silicate
3.	**Action of heat:** Asmall amount of the sample is taken in a dry test tube and heated gartly at first and then strong.	White fumes evolved	Presence of carbonate
4.	**Flame test:** A small amount of the sample is made into a paste with con.HCL in a watch glass and introduced into non luminous part of the Bunsen flame.	Bluish green flame not appeared.	Absence of copper
5.	**Ash test:** A filter paper is soaked into a mixture of sample and cobalt nitrate solution and introduced into the Bunsen flame and ignited	No yellow colour flame	Absence of sodium

Preparation of extract:

5 gm of palagarai chunnam is weighed accurately and placed in a 250ml clean beaker and added with 50 ml of distilled water. Then it is boiled well for about 10 minutes. Then it is cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water.

S.NO	EXPERIMENT	OBSERVATION	INFERENCE
	TEST FOR ACID RADICALS		
1.	**Test for sulphate:** 2ml of the above prepared extract is taken in a test tube to this added 2ml of 4% ammonium oxalate solution.	No cloudy appearance present.	Absence of sulphate.
2.	**Test for chloride:** 2ml of the above prepared extract is added with diluted HNo3 till the effervescence ceases. Then 2 ml of silver nitrate solution is added.	No cloudy appearance present.	Absence of chloride.
3.	**Test for phosphate:** 2ml of the extract is treated with 2ml of ammonium molybdate solution and 2ml of con. Hno3.	No cloudy yellow appearance present.	Absence of phosphate.
4.	**Test for carbonate:** 2ml of the extract is treated with 2ml magnesium sulphate solution.	Cloudy appearance present.	Presence of carbonate.
5.	**Test for sulphide:** 1gm of the substance is treated with 2ml of con HCL.	No rotten egg smelling gas evolved.	Absence of sulphide.
6.	**Test for fluoride and oxalate:** 2ml of extract is added with 2ml of dil.Acetic acid and 2ml calcium chloride solution and heated.	No cloudy appearance.	Absence of fluoride and oxalate.

7.	**Test for nitrite:** 3 drops of the extract is placed on a filter paper, on that 2drops of acetic acid and 2 drops of benzidine solution is placed.	No characteristic changes.	Absence of nitrite.
8.	**Test for borate:** 2 pinches of the substance is made into paste by using sulphuric acid and alcohol (95%) and introduced into the blue flame.	Bluish green colour flame not appeared.	Absence of borate.
II. Test for basic radicals			
1.	**Test for lead:** 2ml of the extract is added with 2ml of potassium iodide solution.	No yellow precipitate is obtained.	Absence of lead.
2.	**Test for copper:** One pinch of substance is made into paste with con Hcl in a watch glass and introduced into the non luminuous part of flame.	Blue colour flame not appeared.	Absence of copper.
3.	**Test for aluminium:** 2ml of the extract sodium hydroxide is added in drops to excess	Yellow colour appeared	Presence of aluminium
4.	**Test for iron:** 2ml of extract add 2ml of ammonium thiocyanate solution and 2ml of con HNO3 is added	Mild red colour appeared	Presence of iron
5.	**Test for zinc:** 2ml of the extract sodium hydroxide solution is added in drops to excess.	White precipitate is formed.	Presence of zinc

6.	**Test for calcium:** 2ml of the extract is added with 2ml of 4% ammonium oxalate solution.	Cloudy appearance and white precipitate obtained	Presence of calcium
7.	**Test for magnesium:** 2ml of extract sodium hydroxide solution is added in drops to excess.	White precipitate is obtained	Presence of magnesium
8.	**Test for ammonium:** 2ml of extract few ml of nessler's reagent and excess of sodium hydroxide solution are added.	No brown colour appeared	Absence of ammonium
9.	**Test for potassium:** A pinch of substance is treated with 2ml of sodium nitrite solution and then treated with 2ml of cobalt nitrate in 30% glacial acetic acid.	Yellowish precipitate is obtained	Presence of potassium
10.	**Test for sodium:** 2 pinches of the substance is made into paste by using HCL and introduced into the blue flame of Bunsen burner.	No yellow colour flame appeared	Absence of sodium
11	**Test for mercury**: 2ml of the extract is treated with 2ml of sodium hydroxide solution	No yellow precipitate is obtained	Absence of mercury
12.	**Test for arsenic:** 2ml of the extract is treated with 2ml of sodium hydroxide solution.	No brownish red precipitate is obtained	Absence of arsenic

III. Miscellaneous			
1.	**Test for starch:** 2ml of extract is treated with weak iodine solultion.	Sky blue colour developed	Presence of starch
2.	**Test for reducing sugar:** 5ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes are noted.	No brick red colour developed	Absence of reducing sugar
3.	**Test for the alkaloids:** a. 2ml of the extract is treated with 2ml of potassium iodide solution. b. 2ml of extract is treated with 2ml of picric acid. c. 2ml of the extract is treated with 2ml of phosphor tungstic acid.	Yellow colour developed	Presence of alkaloids
4.	**Test for tannic acid:** 2ml of extract is treated with 2ml of ferric chloride solution.	No black precipitate is obtained	Absence of tannic acid
5.	**Test for unsaturated compound:** 2ml of extract 2ml of potassium permanganate solution is added.	Potassium permanganate is not decolourised.	Absence of unsaturated compound.
6.	**Test for amino acid:** 2 drops of the extract is placed on a filter paper and dried well.	Not violet colour developed.	Absence of amino acids.
7.	**Test for type of compound:** 2ml of the extract is treated with 2ml of ferric chloride solution.	Red colour developed.	Anti pyrine, aliphatic amino acids and meconic acid are present.

4.4 QUANTITATIVE ANALYSIS

4.4.1 TRACE ELEMENTS ANALYSIS OF PALAGARAI CHUNNAM:

The analysis of heavy metals and trace elements were estimated by using Inductively coupled plasma optical emission spectrometry (ICP- OES). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

ICP-OES:

Perkin Elmer Optima 5300DV was used for standard ICP-OES analysis. The optimized operating conditions are given in table 1, the wavelength of analytical lines are given below and the test drug Palagarai chunnam underwent microwave digestion for sample preparation.

ICP- OES Operating Conditions:

Rf frequency: 40 M Hz

Range: 165 – 782 nm

Detection limit: Up to ppm level using SCD detector

4.4.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time.

The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum. For other uses of this kind of technique, see Fourier transform spectroscopy.

The interference pattern obtained from a two beam interferometer as the path difference between the two beams is altered, when Fourier transformed, gives rise to the spectrum. The transformation of the interferogram into spectrum is carried out mathematically with a dedicated on-line computer.

The Perkin Elmer Spectrum1 FT-IR instrument consists of globar and mercury vapor lamp as sources, an interferometer chamber comprising of KBr and mylar beam splitters followed by a sample chamber and detector. Entire region of 450-4000 cm-1 is covered by this instrument. The spectrometer works under purged conditions. Solid samples are dispersed in KBr or polyethylene pellets depending on the region of interest. This instrument has a typical resolution of 1.0 cm-1. Signal averaging, signal enhancement, base line correction and other spectral manipulations are possible.

Instrument details

Model : Spectrum one : FT-IR Spectrometer

Scan Range : MIR 450-4000 cm-1

Resolution : 1.0 cm-1

Sample required: 50 mg, solid or liquid.

ANALYSIS OF PARTICAL SIZE

The particle size of the Palagarai chunnam was determined using High resolution scanning electron microscopy (HR SEM). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

4.4.3 HR SEM :

A scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The SEM analysis is carried out by using FEI-Quanta FEG 200-High Resolution Instrument

Resolution : 1.2 nm gold particle separation on a carbon substrate

Magnification : From a min of 12 X to greater than 1,00,000 X.

Calculation of the particle size:

The horizontal line in the right corner of the micrograph corresponds to micron in length would be given. A comparison could be made between the length of the particles visible in the micrograph with this line and the length of the particle was calculated.

Application:

To evaluate grain size, particle size distributions, material homogeneity and intermetallic distributions.

4.5 TOXICITY STUDIES OF PALAGARAI CHUNNAM

Preclinical safety evaluation Palagarai chunnam with acute and subacute toxicity study carried out as followed

Principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of animals and the study design. Institutional Animal Ethics Committee approval number: **NIS/IAEC/II/08/2016** dated 29.9.2016 For acute toxicity study and repeated dose 28-day oral toxicity study.

4.5.1 ACUTE TOXICITY STUDY OF PALAGARAI CHUNNAM

Experimental Animals:

Species	: Wistar Albino Rats
Sex	:Female
Age/weight	:6 weeks/140-160gm b.wt
Acclimatization Period	:7 days prior to dosing
Housing	:Polypropylene cages bedding with husk
Husbandry	:12-h light/12-h dark cycle/ Room temperature 22°C ± 3°C and Relative humidity 30–70%
Feed and Water	:Rodent pelleted feed RO purified water
Identification	:Animals will be kept in Polypropylene cages and marked

Experimentation Details of Acute Toxicity Study:

Groups/Treatment regimen	:Grouped by randomization
Test Guideline	:OECD-423
Duration of the exposure to test drug	:Once single dose
No of Animals	:3 Female/ group
Control group	:Adjuvant (Milk)
Test groups	:Palagarai chunnam 300,2000 mg/kg b.wt

The Female Albino Rats of weighing 150-200g were obtained from authorized animal breeders of the animal laboratory in TANUVAS, Madhavaram, and Chennai and stocked in the animal house at National Institute of Siddha, Chennai.

Animals were housed in a cage at 22°C ±3°C and relative humidity 30–70% and have free access to standard rat pellet diet. The animals are divided into three groups (Group I, II &III). Each group contains 3 female Wistar albino rats. Group I served as a control and treated with milk.

The remaining two groups were treated with 300mg/kg.b.wt and 2000mg/kg.b**.**wt dosage of Palagarai chunnam by oral route after 12 hrs fasting with free from water. After drug administration behavioral parameters are monitored for the first 4 hours continuously (1/2 hr, 1hr, 2 hr, 3 hr,4 hr) and noted. The animals that the die within this period will be subjected to necropsy. Remaining animals will be weighed and sacrificed under the injection of Pentothal Sodium on the 15^{th} day of the Study period. The toxicological effect was assessed on the basis of mortality.

Route of administration

Oral route was selected because it is the normal route of clinical administration.

Administration of Dose

The animals were fasted (only food was withheld) for 12hrs and weighed prior to dosing. Three animals were used for each step. A single dose of the solution (300,2000mg/kg/b.wt) was consecutively administered by oral gavage using intubation cannula. The food was withheld for another 4hrs after dosing of the drug.

Observations were made and recorded systematically and continuously observed after the substance administration as per the guidelines.

- ½ hour, 1 hour, 2 hours, 4 hours and up to 24 hours observation
- All rats were observed twice daily for 14 days
- Body weight were Calculated weekly once
- Feed & water intake were Calculated daily

Cage side observation

The animals were monitored for behavioral parameters like Alertness, Aggressiveness, piloerection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, Mortality.

Gross necropsy:

At the end of the 14 th day, all the animals were sacrificed by using the injection of Pentothal sodium Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, eye, lungs, heart, spleen, liver, kidneys, adrenals, uterus, of all animals.

4.5.2 28-DAY ORAL TOXICITY STUDY OF PALAGARAI CHUNNAM

Experimental Animals:

Species	:	Wistar Albino Rats
Sex	:	Male and Female
Age/weight at start of test	:	6 weeks/140-160g b.wt
Acclimatization Period	:	7 days prior to dosing
Housing	:	Polypropylene cages bedding with husk
Husbandry	:	12-h light/12-h dark cycle/ Room temperature 22°C ± 3°C and Relative humidity 30–70%
Feed and Water	:	Rodent pelleted feed RO purified water ad libitum
Identification	:	Animals will be kept in Polypropylene cages and numbered

Experimentation Details of Repeated dose 28 days Toxicity Study:

Groups/Treatment regimen	:	Grouped by randomization
Test Guideline	:	OECD-407
Length of exposure to test substance	:	28 days
No of Animals	:	5 Female+5 Male / group
Control group	:	Adjuvant (Milk)
Test group	:	Low dose, mid dose, High dose

The animals weighted in range of 150-200 gm of 20 male and 20 female Wistar albino rats are used for 28 days repeated oral toxicity study. The animals are divided into four groups. Each group contains 10 animals (5 female and 5 Male). The first group treated as control and second, third, fourth groups were treated with Group 1.Control - Milk, Group 2.Low dose -11.7mg/kg/b.wt, Group 3.Mid dose -58mg/kg/b.wt, 4.Group .High dose.- 117mg/ kg/b.wt .

Above mentioned dose of palagarai Chunnam mixed with milk for 28 days respectively after 12 hrs. of fasting with free from water. The low dose, mid dose and high dose of test drug will be calculated from human therapeutic dose based on surface area by using the conversion table of Paget and Barnes (1964). The control animals were administered with milk as adjuvant.

The administration was given by oral, once daily for 28 consecutive days. The animals were observed the behavioral parameters for the study period. Body weight of the animal was being monitored at weekly intervals. Food & water intake were Calculated daily. All the animals were sacrificed at the end of the study (29 days) by using the intra peritoneal injection of Pentothal Sodium as prescribed dose level. Blood was collected from the anesthetized animals from the Abdominal aorta for the following investigations like Hematology and Biochemical analysis. Gross pathological changes were monitored in all the organs then the vital organs were preserved and subjected to Histopathological examination.

Observation:

Experimental animals were kept under observation throughout the course of study for the following

- All rats were observed twice daily for 28 days
- Body weight were Calculated weekly once
- Feed & water intake were Calculated daily

a.Cage side observation

The animals were monitored for behavioral parameters like, Alertness, Aggressiveness, piloerection, Grooming, Gripping, Touch Response, Motor Activity, Tremors, Convulsions, Muscle Spasm, Catatonia, Muscle relaxant, Hypnosis Analgesia, Lacrimation, Exophthalmos, Diarrhea, Writhing, Respiration, Mortality.

Laboratory Investigations:

On the 29^{th} day, the animals were fasted overnight, then anesthetized to collect blood samples from the abdominal aorta in two tubes: one with EDTA for hematological parameters, another one without any anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum for biochemical parameters.

Hematological Investigations:

Blood samples of control and experimental rats were analyzed for haemoglobin (Hb), total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Platelet, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), were calculated by auto analyzer.

Biochemical Investigations:

Serum samples of control and experimental animals were analyzed for, Bilirubin, BUN, Creatinine, Triglyceride, Total Cholesterol, HDL, LDL, VLDL, using standard methods. Activities of glutamate oxaloacetate transaminase/Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine aminotransferase (GPT/ALT) were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on the 29^{th} day and satellite group were sacrificed on after 14 days. Gross necropsy includes examinations of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents. Brain, eye, lungs, heart, spleen, liver, kidneys, adrenals, sex organs, of all animals were recorded.

Histopathology:

The organs included liver, kidneys, spleen, brain, heart, lungs and stomach of the animals were preserved, and they were subjected to Histopathological examination.

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of all the animals (low, mid, high) a n d satellite group w e r e preserved and fixed in 10% formalin for 24 hrs. Samples were dehydrated in an auto technic and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at $50^{O}C$ and then in a cubical block of paraffin made by the "L" molds. It was followed by microtome and the slides were Prepared then stained with Haematoxylin-eosin.

Statistical analysis:

Findings such as body weight changes, food consumption, water intake, and hematology and biochemical analysis were subjected to One-way ANOVA Dunnet's test using a computer software program followed by **D *Graph Pad Instat-3***

5. RESULTS

Standardization of Chunnam in Siddha Aspect

A small amount of turmeric powder was taken. Then same amount of Palagarai chunnam is mixed with turmeric powder, while adding water, the colour of the mixture was turned into red colour[34].

1.Turmeric Powder

2-Palagarai chunnam and turmeric powder

3. Chunnam confirmation

5.1 QUALITATIVE ANALYSI S

Table 1: Organoleptic analysis of –Palagarai chunnam

S.no	Parameters	Results	Method of Testing
1.	Colour	Dull white	By visual
2.	Odour	Odourless	Olfactory examination
3	Nature	Fine powder	By visual

From Table 1, The Organoleptic characters shows that Palagarai chunnam is dull white in color and odorless powder

Table 2: Physiochemical analysis of –Palagarai chunnam

S.no	Parameters	Percentage
1	Loss on drying	Less than 1%
2	Solubility	Soluble in water& alcohol.
3	pH At 25 ^{0}C (1% w/w solution)	7.87%
4	Total ash value	97.01%
5	Acid insoluble ash	34.69%
6	Water soluble ash	16.96%
7	Water soluble extraction	Less than 1%
8	Alcohol soluble extraction	Less than 1%

From Table 2, The Physico-chemical analysis of Palagarai chunnam explained in the parameters such as Moisture content, Total ash value, Acid insoluble ash, Water soluble ash, Water soluble extraction, Alcohol soluble extraction and pH are within the normal limits. The drug with pH of 7.87%. It is easily soluble in water, alcohol and acetone and ether.

Table 3: Biochemical analysis of Palagarai chunnam

Test for Basic radicals

S.no	Procedures	Palagarai chunnam
1.	Test for Lead	-
2.	Test for Copper	-
3.	Test for Aluminum	+
4.	Test for Ferrous iron	+
5.	Test for Zinc	+
6.	Test for Calcium	+
7.	Test for Magnesium	+
8.	Test for Ammonium	-
9.	Test for Potassium	+
10.	Test for Arsenic	-
11.	Test for Mercury	-
12	Test for Sodium	-

The table no:3 explained that presence of Aluminum, Ferrous iron, Zinc, Calcium, Magnesium in the test drug.

Table-4: Test for Acidic radicals

S.no	Procedures	Palagarai chunnam
1.	Test for Sulphate	+
2.	Test for Chloride	+
3.	Test for Phosphate	_
4	Test for Carbonate	+.
5.	Test for Sulphide	_
6.	Test for Fluoride and oxalate	_
7	Test for Nitrate	_
8	Test for borate	_

Table-5: Test for Miscellaneous

S.no	Procedures	Palagarai chunnam
1.	Test for Starch	+
2.	Test for Reducing sugar	_
3.	Test for Alkaloids	+
4.	Test for Amino Acids	_
5.	Test for Tannic acids	_
6	Test for type of compound	Anti pyrine, aliphatic amino acids and meconic acid are present.

5.2 QUANTITATIVE ANALYSIS

A .ICP-OES:

Sample Weight of test drug: **0.35300gm**

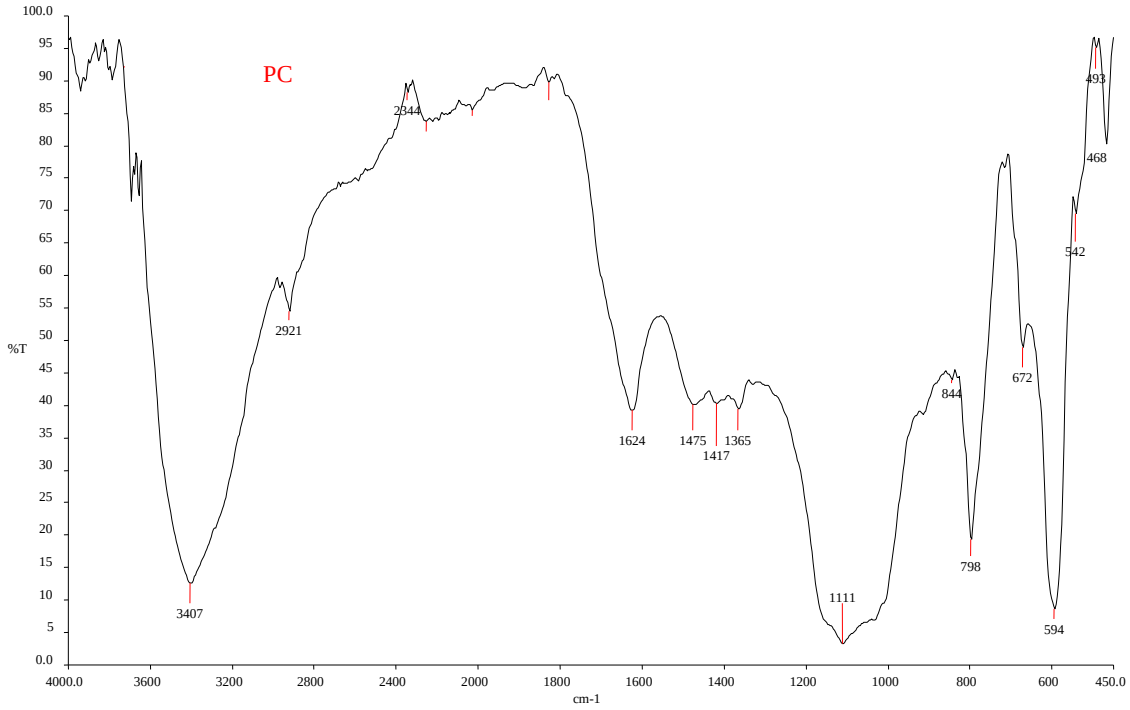
Table no:6 Result of Quantitative analysis by **ICP-OES** for Palagarai chunnam

S.No:	ELEMENTS	WAVE LENGTH IN NM	RESULTS (in mg/L)
1.	Aluminium	Al 396.152	30.213
2.	Arsenic	As 188.979	BDL
3.	Calcium	Ca 315.807	625.150
4.	Cadmium	Cd 228.802	BDL
5.	Copper	Cu 327.393	BDL
6.	Iron	Fe 238.204	45.016
7.	Mercury	Hg 253.652	BDL
8.	Potassium	K 766.491	3.004
9.	Magnesium	Mg 285.213	1.004
10.	Sodium	Na 589.592	BDL
11.	Nickel	Ni 231.604	BDL
12.	Lead	Pb 220.353	BDL
13.	Phosphorus	P 213.617	38.541
14.	Sulphur	S 180.731	45.224
15.	Zinc	Z 206.200	10.018

***BDL – Below Detection Limit**

B. FT-IR Analysis of Palagarai chunnam

Graph-1: Graph of Characteristic IR absorption frequencies of Organic Functional Groups for unpurified raw drug Palagarai.

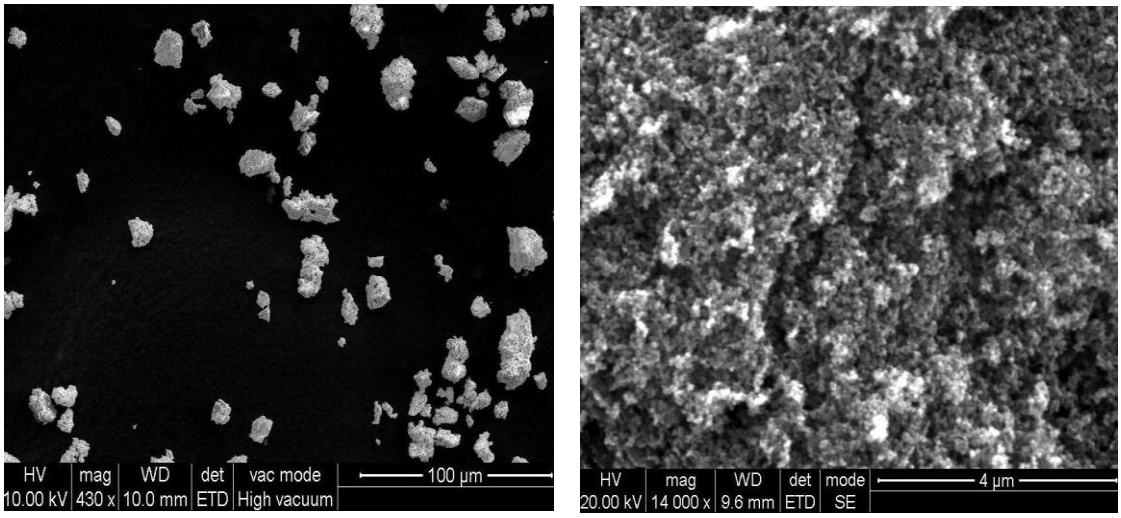


Fourier Transform Infra Red Spectroscopy (FTIR) analysis of Palagarai chunnam shows the presence of vibrational band observation around ~ 1420 to 1500 cm^{-1} and 860 to 875 cm^{-1} confirms is attributed to the presence of calcium carbonate. The FTIR results shows the observed N-H stretch, O-H stretch, H-C-H stretch, C=O stretch, N-H stretch, C-C=C symmetric stretch, H-C-H bend, C-O stretch, C -H bend, C-C stretch which indicates that the presence of functional groups Amide, Phenols and alcohols, Alkanes, Aldehyde, Amine, Alkenes, Alkanes , Ester , ether, Alkyne.

C. SCANNED ELECTRON MICROSCOPY

Determination of Particle size of Palagarai chunnam

Figure-1: SEM Image of Palagarai chunnam



The morphology of the Palagarai chunnam drug can be determined by SEM (FEI Quanta). A representative portion of each sample must be sprinkled onto a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination. We have observed from SEM photographs that particles are spherical in shapes and sizes are in the range from **1 micron to 3 microns**. Although the particle sizes of different batches showed similarity, it seems that these particles are aggregates of much smaller particles. When dispersed in an aqueous medium, these preparations form a negatively charged hydrophobic particle suspension. This hydrophobicity gives these particles a tendency to aggregate together to form larger particles. Palagarai Chunnam exhibited larger sizes and agglomeration of the particles. Therefore, the comparatively larger size may be due to the agglomeration of the particles by repeated cycles of calcinations involved in preparation

1. ACUTE TOXICITY STUDY

Table.7 Behavioral Signs of Acute Toxicity Study

No	Dose Mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	Control	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2.	300	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
3.	2000	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9.Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14.Analgesia 15.Lacrimation 16. Exophthalmos 17.Diarrhoea 18.Writhing 19.Dyspnoea 20. Mortality

Note:

+ Presence of activity

- Absence of activity

All the data were summarized in the form of (table) revealed that there was no abnormal signs and behavioral changes in all animals at the dose level of **300,2000 mg/kg** body weight administered orally, during the study period.

There was no mortality observed after dosing of **Palagarai chunnam** upto 2000mg/kg body weight This indicates that LD50 of **Palagarai chunnam** is more than 2000mg/kg b.wt.

There were no changes in skin and fur, eyes and mucous membranes of all animals. The eating, drinking habit, sleep pattern, locomotion were normal in all animals and no changes in body weight as compared to control group. At the end of the 14^{th} day, necropsy was performed and there was no abnormality seen in test groups as compared to control group during the examination.

28 DAY REPEATED DOSE ORAL TOXICITY STUDY (FOOD CONSUMPTION)

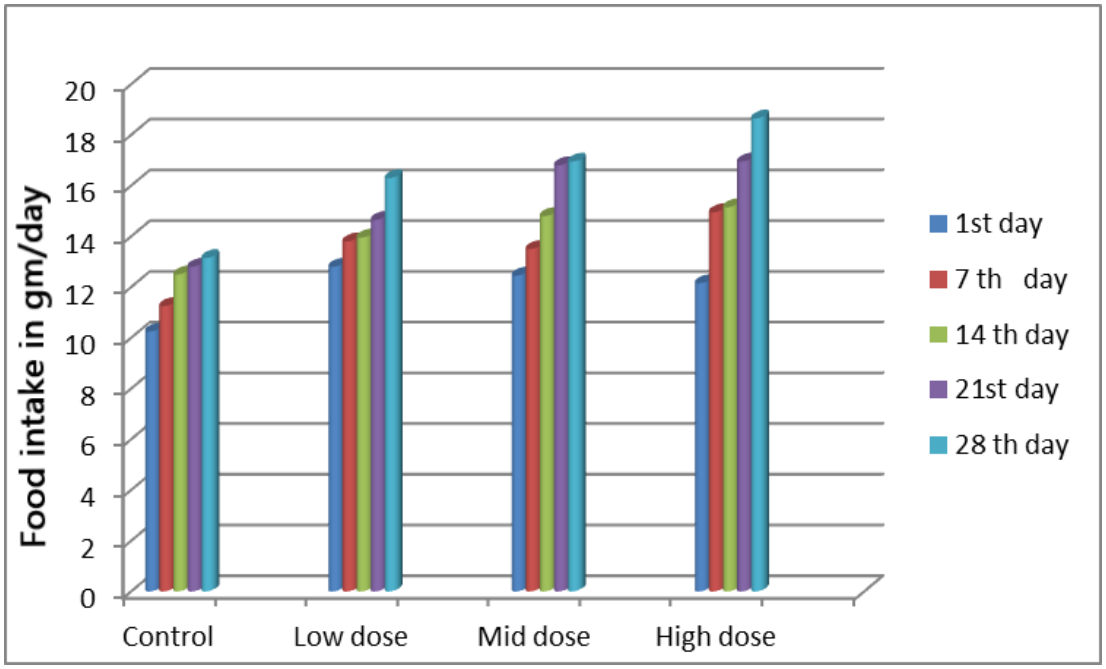
Food consumption of the animals significant difference in Food intake the test group animals were observed when compared with control group during the study period. (Table 8), There was significant difference occurs in test groups compared with control group. but they are within physiological limit.

Table 8: Food (g/day) intake of albino rats exposed to Palagarai chunnam

Dose (mg/kg/ day)	1st day	7 th day	14 th day	21st day	28 th day
Control	10.25±0.16	11.25±0.18	12.5±0.28	12.8±0.25	13.15±0.18
LD	12.8±0.21**	13.8±0.21**	13.95±0.38*	14.65±0.38**	16.3±0.32**
MD	12.45±0.16**	13.5±0.45**	14.8±0.54**	16.8±0.54*	16.95±0.15**
HD	12.15±0.158**	14.95±0.17**	15.15±1.25**	16.95±0.71*	18.65±0.25**

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05, **P<0.01.

Food (g/day) intake of albino rats Exposed to Palagarai chunnam- Figure 4



28 DAY REPEATED DOSE ORAL TOXICITY STUDY (WATER CONSUMPTION)

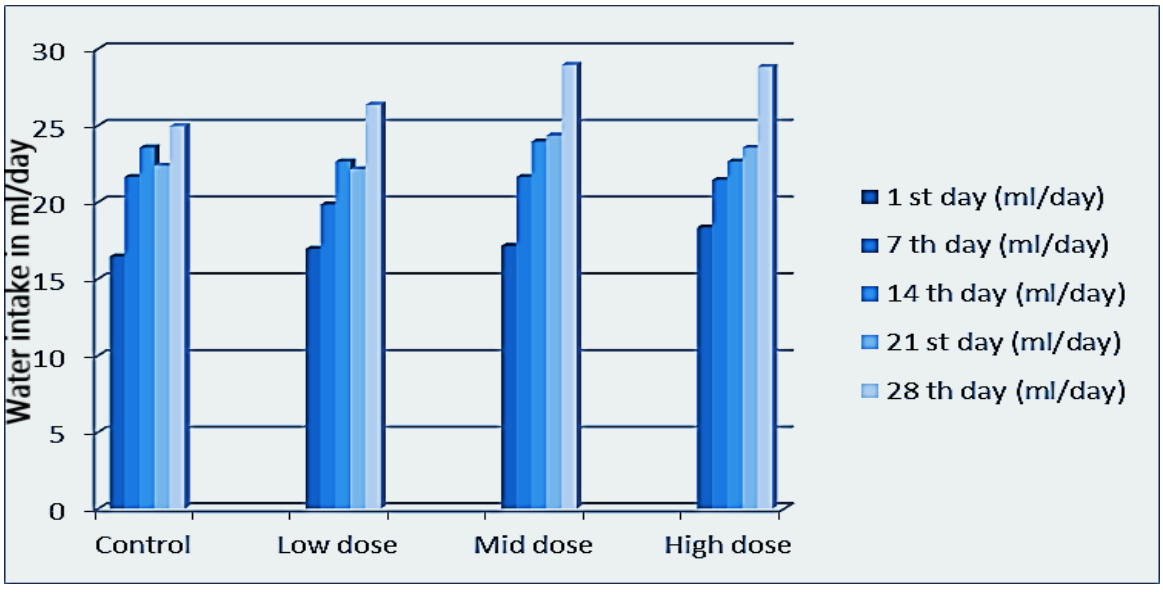
Water consumption the difference in Water intake of control and test group of animals observed during the study period. There was significant difference occurs in the test group compared with control group (Table 9)**.**

Table: 9 Water (ml/day) intake of albino rats exposed to Palagarai chunnam

Dose (mg/kg/day)	1 st day	7 th day	14 th day	21 st day	28 th day
Control	16.45±0.35	21.65±0.45	23.56±0.38	22.38±0.25	24.95±0.95
LD	16.95±0.25	19.84±0.35**	22.65±0.15*	22.15±0.18	26.35±0.25**
MD	17.15±0.35	21.65±0.35	23.95±0.18	24.35±0.28*	28.95±0.28**
HD	18.36±0.46	21.45±0.24	22.65±0.28*	23.55±0.38**	28.83±0.24**

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05,**P<0.01

Water (ml/day) intake of albino rats Exposed to Palagarai chunnam-Figure 5



28 DAY REPEATED DOSE ORAL TOXICITY STUDY-BODY WEGHT

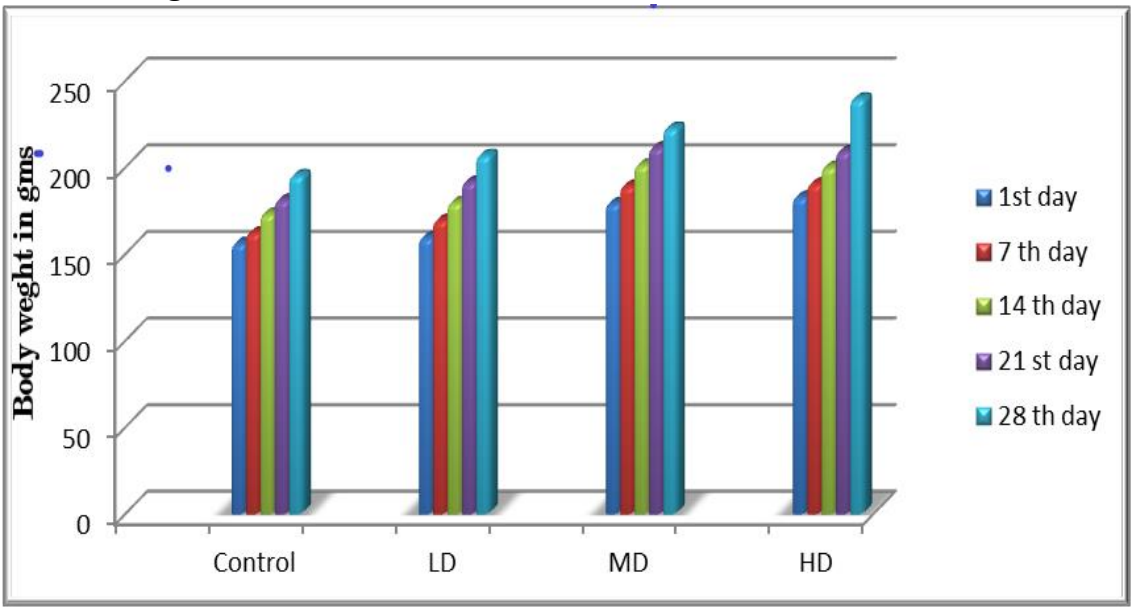
Body weight of both control and test dose group revealed normal body weight throughout the study .There is significant change occur in body weight of mid and high dose group compared with control group (Table 10).

Table:10 Body weight (g) changes of albino rats (male) exposed to Palagarai chunnam

Dose Mg/kg	1st day	7 th day	14 th day	21 st day	28 th day
Control	155±12.70	161±13.50	172±15.20	180.66±18.45	194±19.20
LD	158.2±10.05	168.3±14.07	178.45±16.52	190.33±18.71	205.66±18.45
MD	178±11.50**	188±12.51**	200.23±12.36*	210.23±15.6*	221.33±20.64
HD	181.66±7.50*	189.33±8.02*	199±9.21	208±8.29*	238.33±10.50**

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05,**P<0.01

Body weight (gm.) changes of albino rats (male) exposed to Palagarai chunnam-Figure -6



28 DAY REPEATED DOSE ORAL TOXICITY STUDY- BODY WEGHT

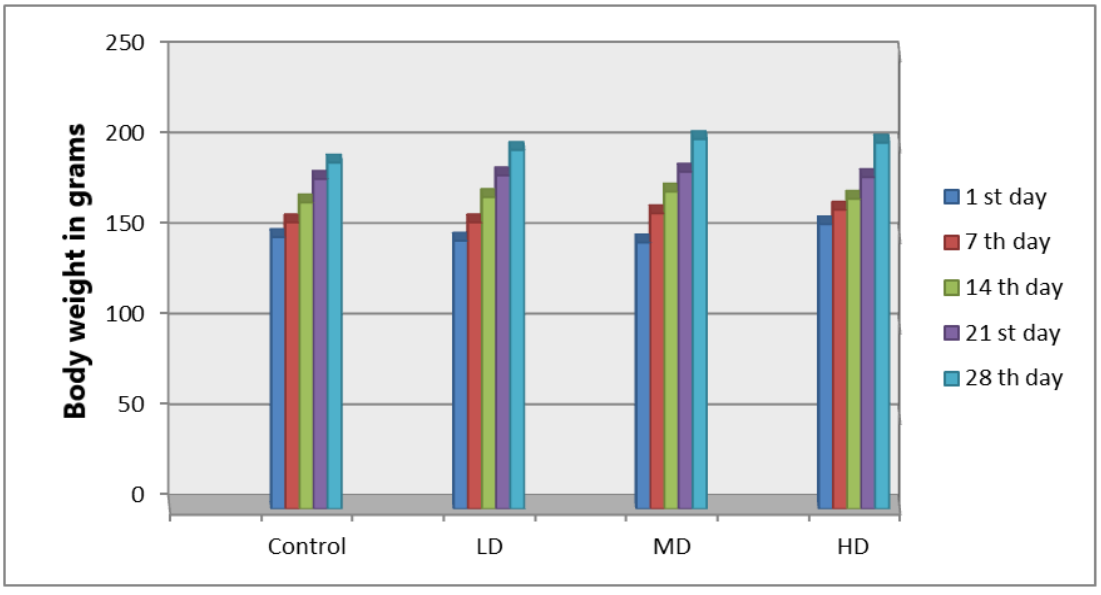
Body weight of both control and test dose group exhibited normal body weight throughout the study period. (Table 11)

Table:11 Body weight (g) changes of albino rats (female) exposed to Palagarai chunnam

Dose (mg/kg/day)	1 st day	7 th day	14 th day	21 st day	28 th day
Control	148±4.55	154.66±4.66	164±5.35	174.33±8.08	186.33±6.78
LD	143.23±5.14	152.66±6.09	165.66±8.09	176±8.18	192.33±6.02
MD	147.66±6.09	157.35±6.68	169.56±6.85	181.65±5.15	194.33±8.72
HD	152.66±5.85	159.66±6.80	164.15±6.25	177±5.55	195.35±7.15

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05,**P<0.01

Body weight (g) changes of albino rats (female) exposed to Palagarai chunnam -Figure-7



28 Day Repeated Dose Oral Toxicity Study

The results of the **Hematological investigations** conducted at the end of the study, the test groups revealed slightly significant changes in levels of hematological parameters, when compared with control group. (Table12)

Table: 12 Effect of Palagarai chunnam on Hematological Parameters

	CONTROL	LD	MD	HD
RBC ($\times 10^6$ µl)	5.68±0.35	6.02±0.52*	6.09±0.57	6.18±0.91
WBC ($\times 10^3$ µl)	9.6±1.42	8.37±1.39	8.5±2.85	7.78±2.16
PLT ($\times 10^3$ µl)	768.5±89.14	685.5±142.54	583.5±94.52**	765.7±96.55
HGB (g/dl)	11.64±1.69	12.16±1.37	13.2±1.23	13.8±1.59**
MCH (pg)	18.39±2.47	18.87±1.71	20.97±0.87**	22.41±1.46**
MCV (fl)	56.98±2.73	59.81±3.81	58.86±4.36	59.7±5.76
Neutrophils$10^3 mm^3$	2±0.52	1.91±0.42	1.41±0.33**	1.52±0.23*
Eosinophil's (%)	1.58±0.22	1.41±0.24	1.44±0.19	1.54±0.29
Basophils (%)	0.2±0.42	0.2±0.42	0.2±0.42	0.1±0.32
Lymph (%)	68.14±5.41	83.42±7.17**	79.07±9.06**	71.97±7.94
Mon (%)	3.82±0.94	2.59±0.94	4.13±0.79	3.76±1.02

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05,**P<0.01

FIGURE :8

Effect of Palagarai chunnam on Hematological Parameters-RBC

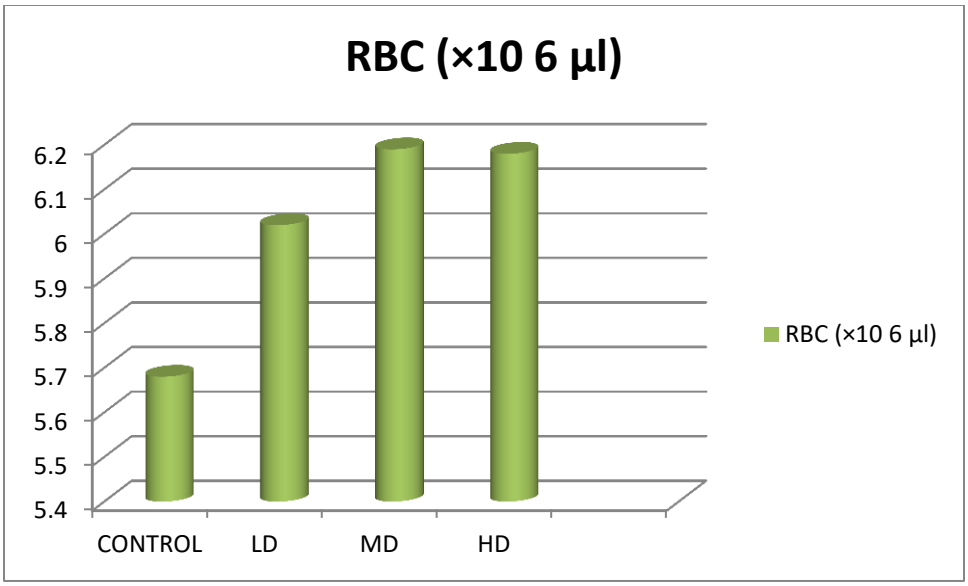


FIGURE :9

Effect of Palagarai chunnam on Hematological Parameters-WBC

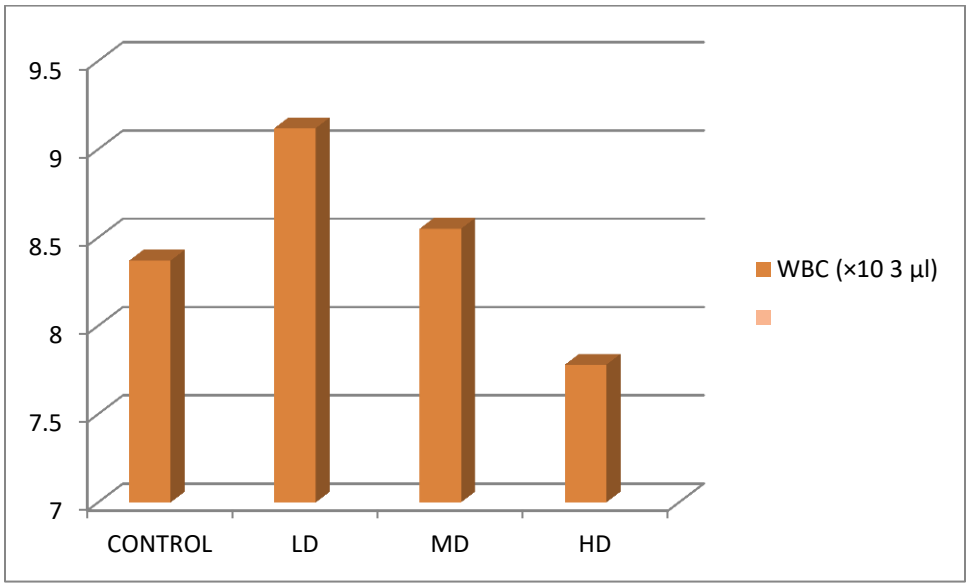


FIGURE :10
Effect of Palagarai chunnam on Hematological Parameters-HEMOGLOBIN

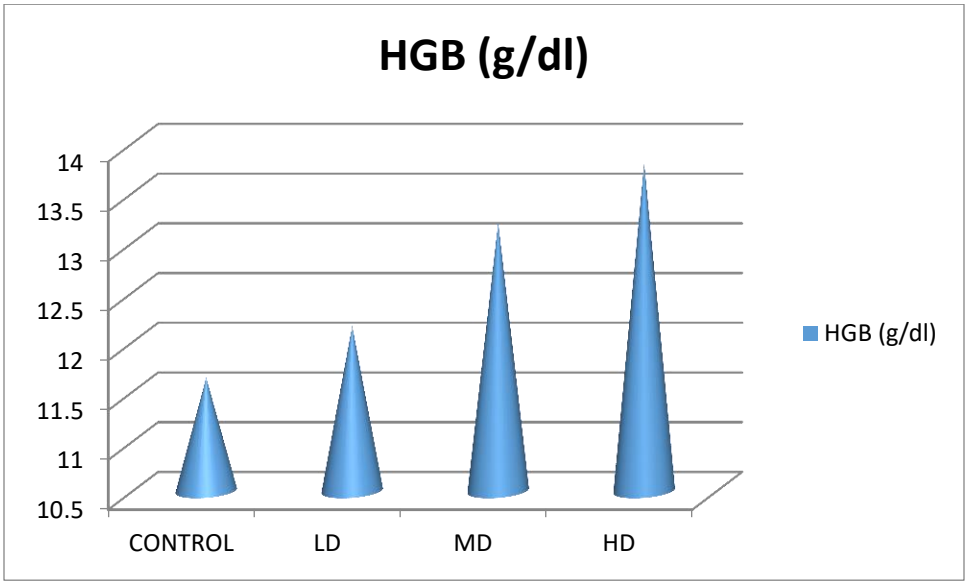
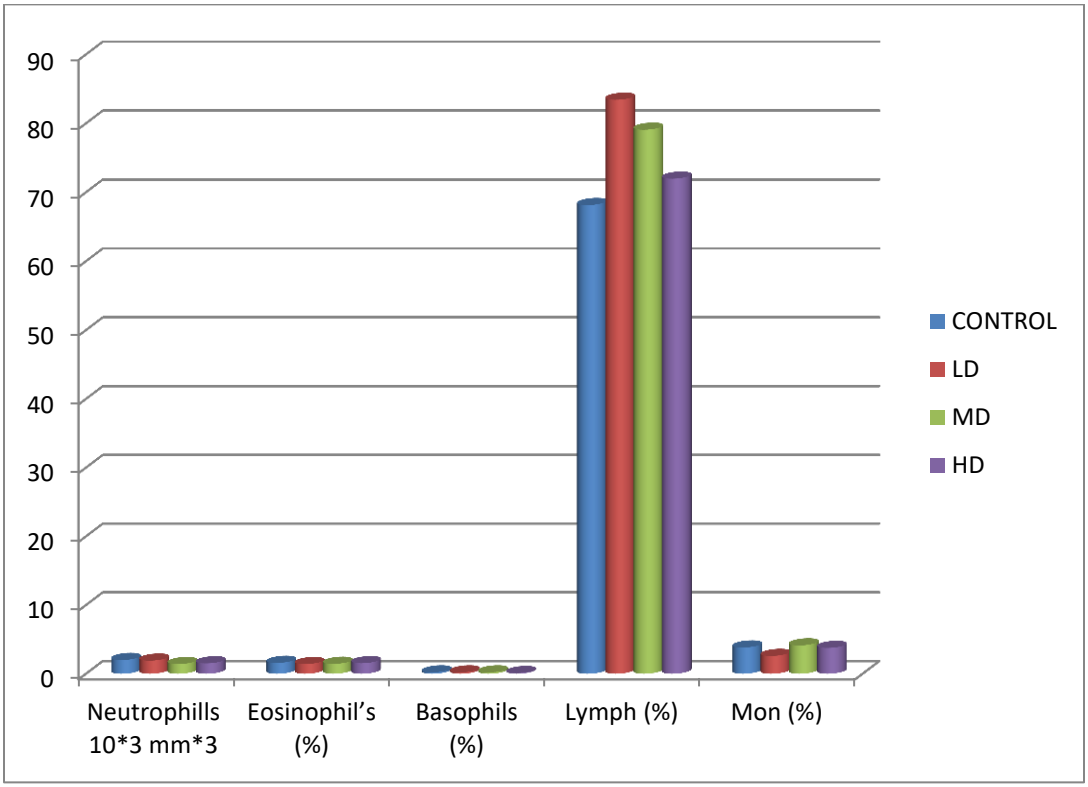


FIGURE :11
Effect of Palagarai chunnam on Hematological Parameters-Differential count



28 Day Repeated Dose Oral Toxicity Study

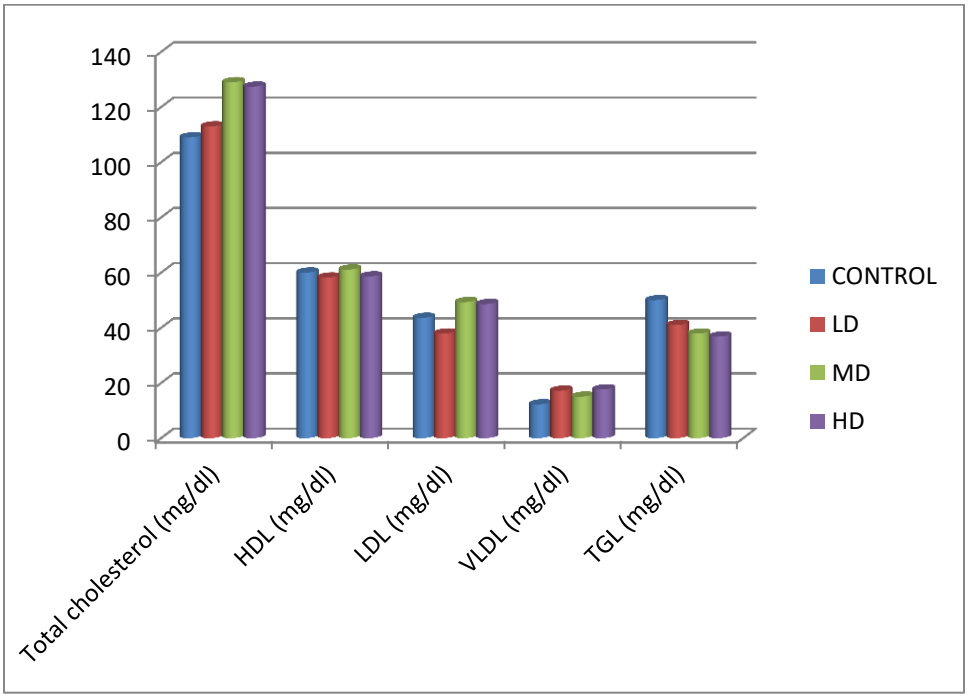
Biochemical investigations were conducted at the end of the study and the results were recorded. In test groups there was significant changes present in biochemical parameters (Lipid profile) , when compared with the control group. At the values were normal biological limits. (Table 13)

Table:13 Effect of Palagarai chunnam on biochemical parameters-Lipid profile

	CONTROL	LD	MD	HD
Total cholesterol (mg/dl)	109.26±15.22	113.93±10.09	129.17±8.41**	127.4±11.56**
HDL (mg/dl)	60±4.74	58.2±8.26	61.1±8.79	58.6±7.95
LDL (mg/dl)	43.6±13.21	37.2±9	49.3±7.01	48.6±11.14
VLDL (mg/dl)	12.25±1.34	17.2±4.57**	15.04±2.54	17.66±4.11**
TGL (mg/dl)	50±4.69	41±10.39	37.9±7.99**	36.8±9.93**

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05,**P<0.01

Effect of Palagarai chunnam on Biochemical parameters –Figure 12



28 Day Repeated Dose Oral Toxicity Study

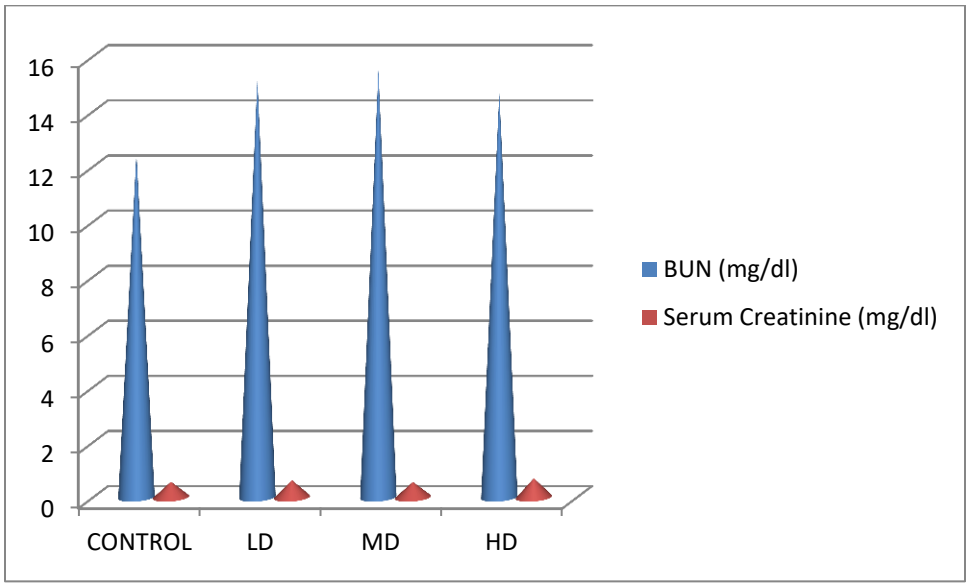
The results of the renal functions test conducted at the end of study, test groups revealed marginally significant changes in levels of renal parameters, when compared with control group.(Table 14)

Table: 14 Effect of Palagarai chunnam on Renal Parameters

	CONTROL	LD	MD	HD
BUN (mg/dl)	12.5±1.58	15.1±2.47*	15.5±2.17**	14.7±2.16
Serum Creatinine (mg/dl)	0.57±0.15	0.63±0.13	0.56±0.18	0.7±0.24

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05,**P<0.01

Effect of Palagarai chunnam on Renal Parameters-–Figure 13



28 Day Repeated Dose Oral Toxicity Study

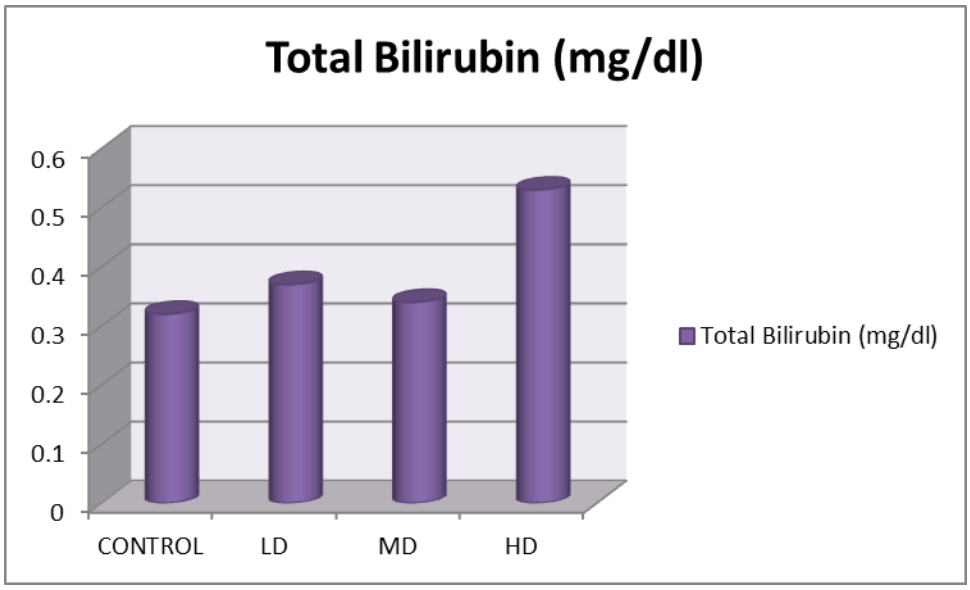
The results of the liver function test conducted at the end of the study, test groups revealed significant changes in levels of liver parameters, when compared with control group.

Table:15 Effect of Palagarai chunnam on Hepatic Parameters

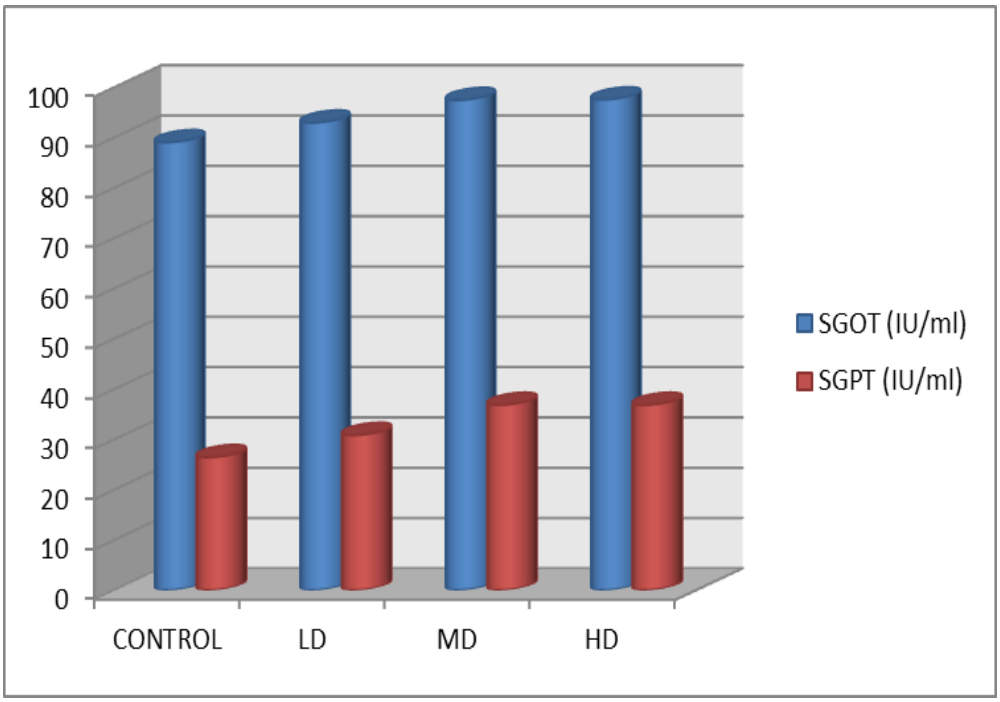
	CONTROL	LD	MD	HD
Total Bilirubin (mg/dl)	0.32±0.12	0.37±0.11	0.34±0.15	0.53±0.38
SGOT (IU/ml)	89±10.82	92.9±17.95	97.4±32.3	97.5±16.15
SGPT (IU/ml)	26.4±7.27	30.8±7.10	36.8±6.97**	36.8±7.05**

Values were expressed as mean± S.D. for N=10 rats in each group one-way ANOVA followed by Dunnett's test. Significant indicates *P<0.05,**P<0.01

Effect of Palagarai chunnam on Hepatic Parameters-–Figure 14



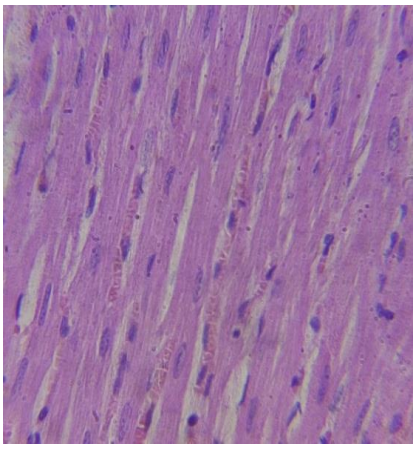
Effect of Palagarai chunnam on Hepatic Parameters-–Figure 15



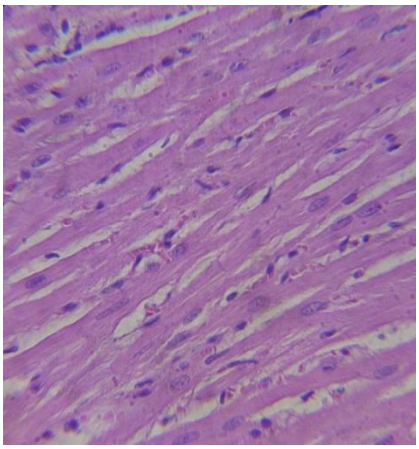
28 Day Repeated Dose Oral Toxicity Study

Histopathology of Heart

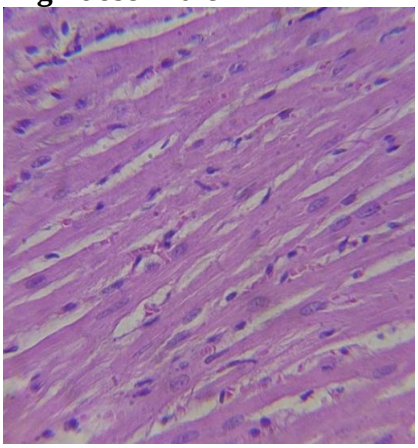
Control –Male



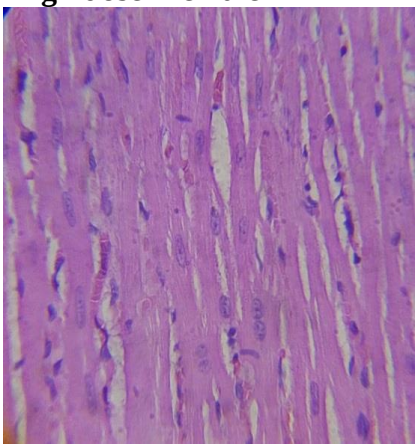
Control- Female



High dose-Male



High dose- Female



Control Male **:** Sarcoplasmic region of myocardium appears normal

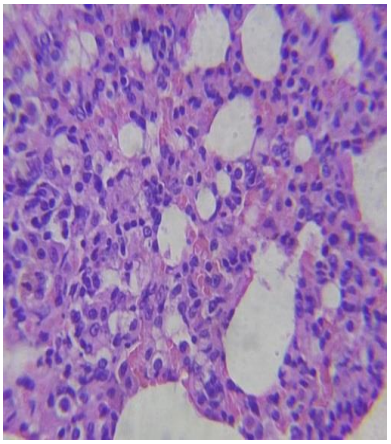
Control Female **:** Endocardium appears normal with no evidence of necrosis

High dose Male **:**Appearance of fibrils and cross striations are equidistant

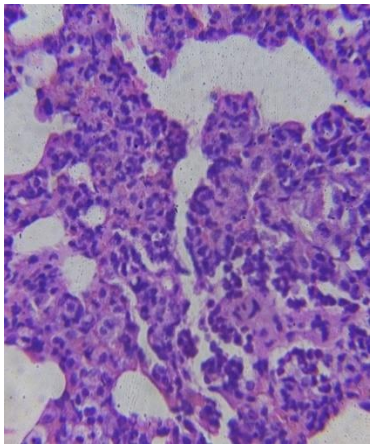
High dose Female : No evidence of atherosclerosis and thrombosis ,No evidence of necrotic myocardium

2.Lungs

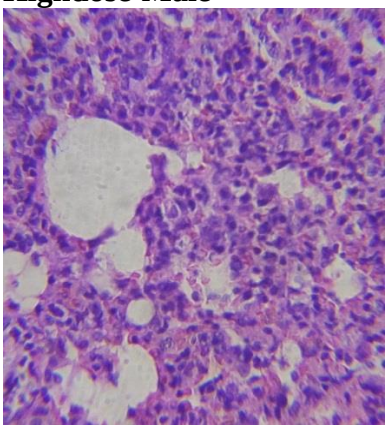
Control-Male



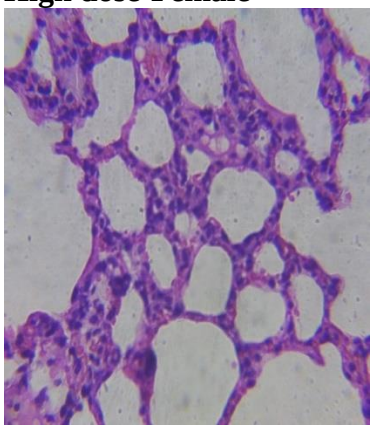
Control-Female



Highdose-Male



High dose-Female



Control Male : Bronchial opening appears regular with no signs of infiltration

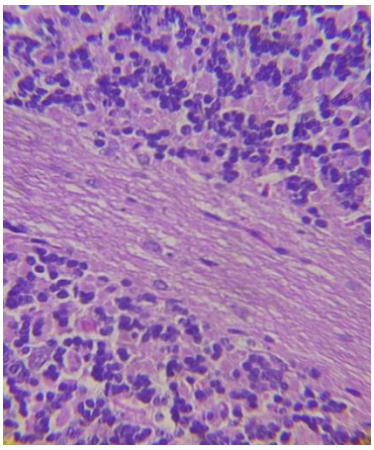
Control Female : Lung parenchyma appears normal with regular arrangement of alveoli and alveolar sac with no signs of lymphocyte infiltration and pulmonary fibrosis

High dose Male : Perfect network of simple squamous epithelium were observed

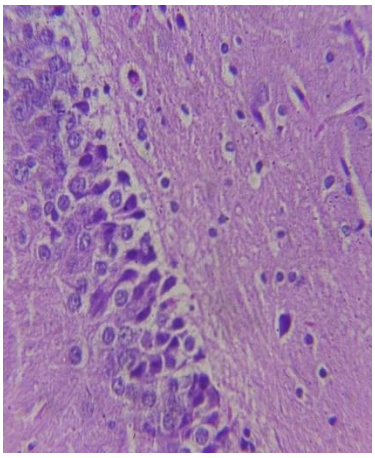
High dose Female: Appearance of alveolar network was normal

3. Brain

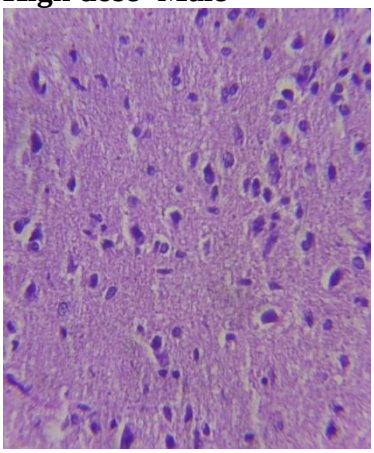
Control-Male



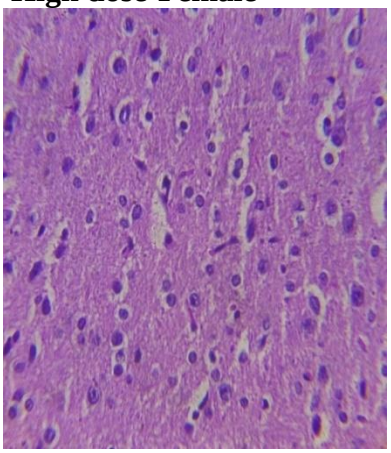
Control- Female



High dose- Male



High dose-Female



Control Male **:** Showed normal architecture in both cortex and medulla where three layers of cerebellar cortex

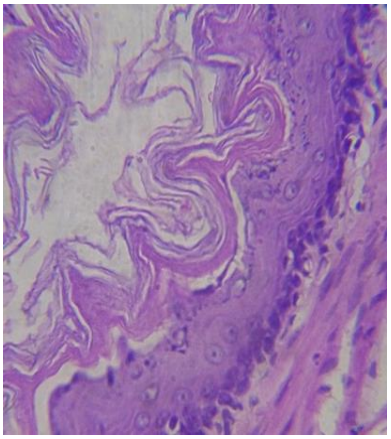
Control Female **:** Appearance of Hippocampal neurons was normal with dense network

High dose Male **:** Regular marginal alignment on the neurons with promising histology were observed

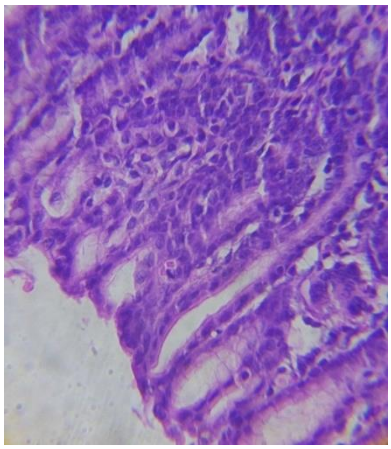
High dose Female: Normal neural architecture with No signs of vascular congestion and edema

3. Stomach

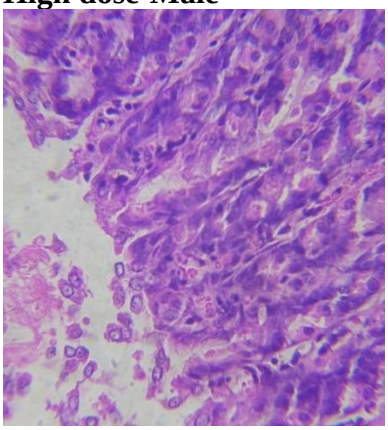
Control-Male



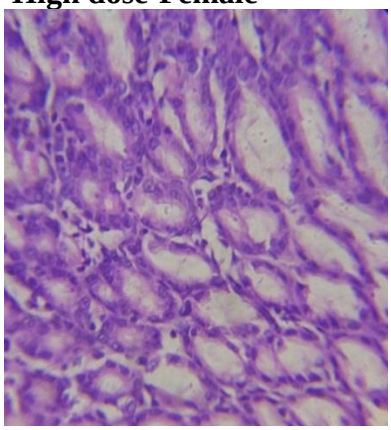
Control- Female



High dose-Male



High dose-Female



Control Male : Regular histology of Inner circular muscle (ICM), gastric pit (GP), and muscularis mucosae (MM) were observed.

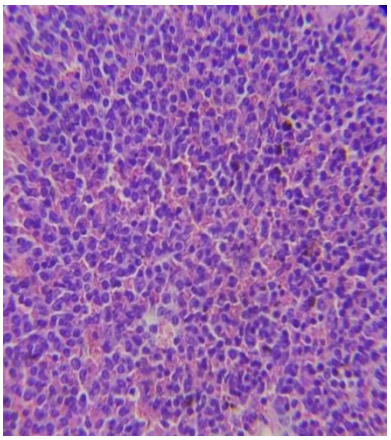
Control Female: Gastric glands, gastric glands including secretary sheath appears normal

High dose Male: The continuity of mucosa was normal with no evidence of ulceration

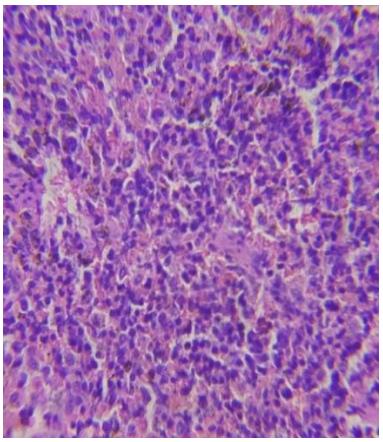
High dose Female: Normal gastric mucosa containing intact gastric glands and parietal cells

4. Spleen

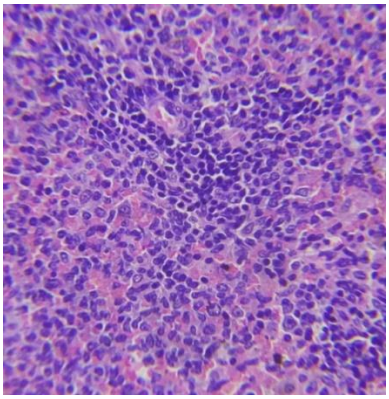
Control-Male



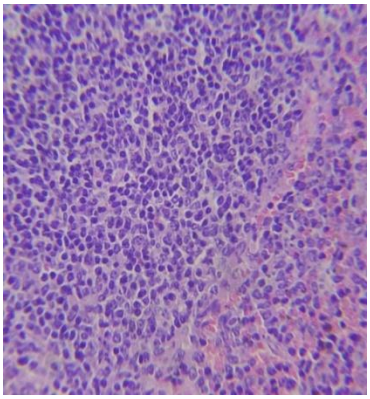
Control-Female



High dose-Male



High dose-Female



Control Male : The appearance of red pulp and marginal sinus are normal. No abnormalities found in lymph nodes

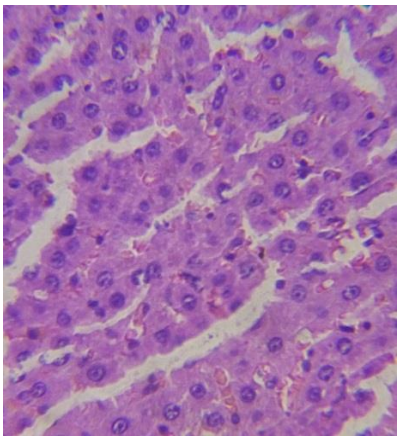
Control Female: Marginal sinus (MS) of the spleen and its sinus lining cells appears normal

High dose Male: Mild changes were observed in spleen architecture with increased number of megakaryocytes.

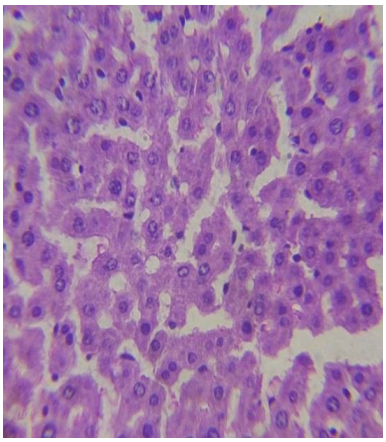
High dose Female: Appearance of splenic red pulp was normal,No signs of perivascular inflammation.

5. Liver

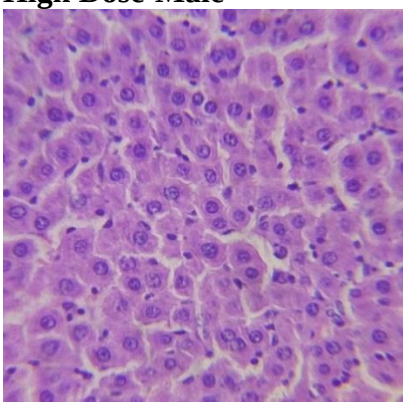
Control-Male



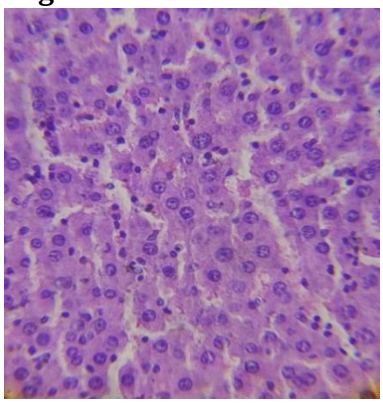
Control-Female



High Dose-Male



High dose-Female



Control Male: No evidence of collagen (fibrosis) increased numbers of kupffer cells were observed and increase in sinusoidal spaces were observed

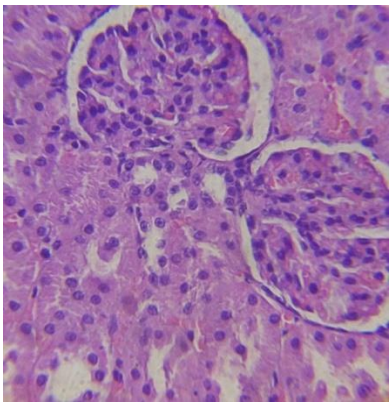
Control Female: Distinct widen hepatic cords that were dorsally radiating from the central vein were observed. Occasional appearances of foamy cytoplasm were observed.

High Dose Male: Liver sinusoid appears widen with occasional bi nucleated hepatocytes

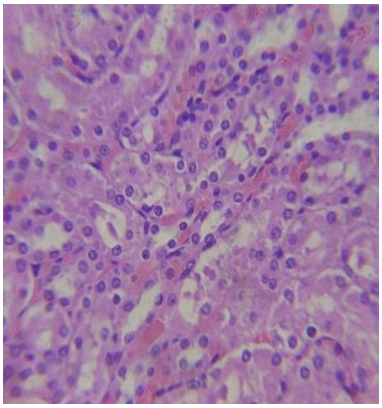
High dose Female: Derangement of hepatic parenchyma with mild congestion of central vein were observed.

6.Kidney

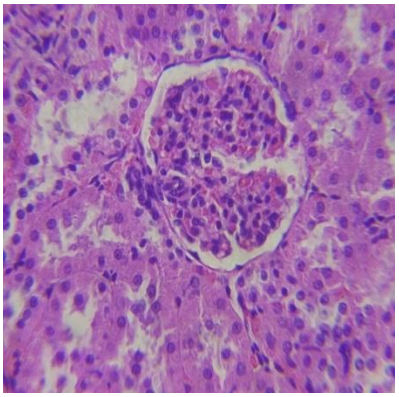
Control –Male



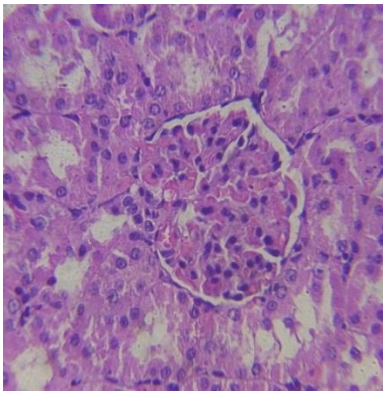
Control-Female



High dose- Male



High dose-Female



Control Male **:** Appearance of glomerular basement membrane was normal, lumen of vessels and bowman's space appears normal

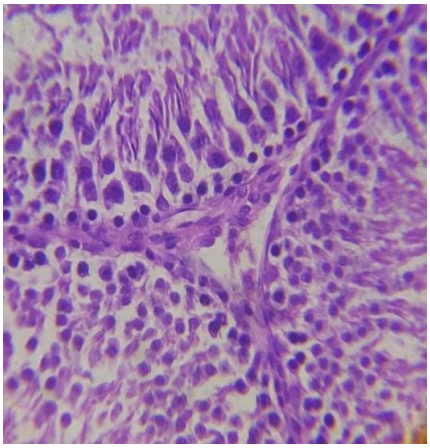
Control Female **:** Section projecting the evidence of tubular protein cast.

High dose Male **:** Arrangement of glomerular loop was normal with regular interstitium

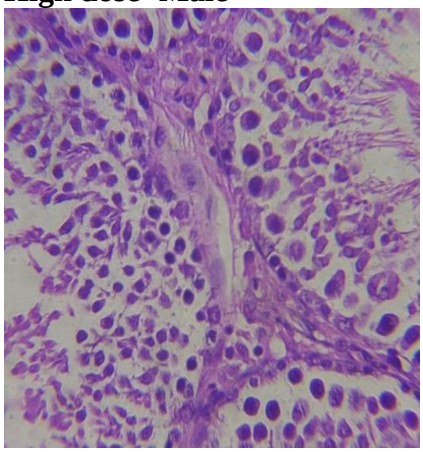
High dose Female: No evidence of interstitial inflammation and lymphocyte accumulation The dilated tubules are lined by flattened epithelium and their lumen

7. Testis

Control- Male



High dose -Male

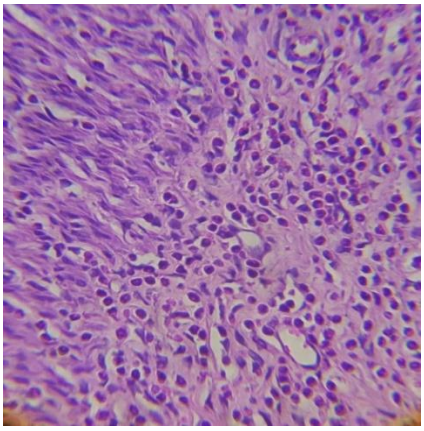


Control Male **:** Histo cytology of testicular tissue shows well differentiated germ cells

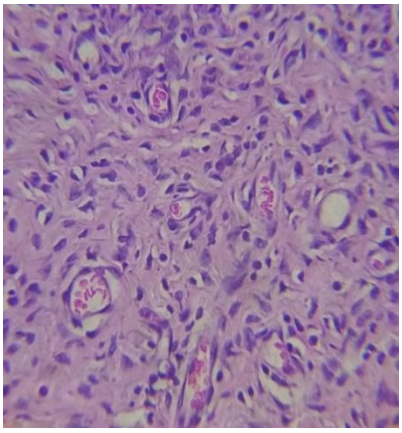
High dose Male : Appearance of leydig cells, interstitial tissue , seminiferous tubule, Sertoli cells and spermatogonia were normal.

8. Uterus

Control-Female



High dose- Female

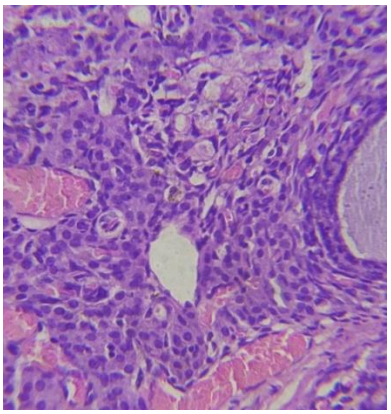


Control Female **:** Endometrial gland, epithelium and blood vessels appears normal

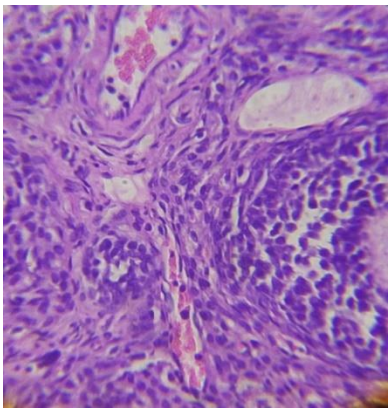
High dose Female : Arrangement of stratum basale, functionale and surface epithelium seems normal

9. Ovary

Control-Female



High dose-Female



Control Female **:** Corpora lutea (CL), atretic follicles (AF) and interstitial tissue (IT) appears normal

High dose Female **:** Sequential arrangement of granulosa cells around oocyte was normal and regular

.

6. DISCUSSION

Metals and minerals are held in hand to hand in Siddha Pharmaceuticals with suitable as well as various process of purification. Since Palagarai obtained from sea, is believed to contain a large number of essential minerals in it. Therefore it has to be purified before using in the medicine preparation. In Siddha system of medicine one of the farfetched drug is palagarai, it has a long history in the treatment of many diseases among Siddha physicians.

Palagarai is used in many area of medicine, it plays vital role in siddha medicinal practice. The usage of palagarai mentioned in siddha literature has to be reviewed. The Medicinal preparation from palagarai are mostly Parpam, Chenduram, Chunnam. Palagarai Parpam is indicated as an antidote to various to types of poisons of animals and other living creatures. Therefore, exploration about this natural resource drug at this time will be economically beneficial as huge amount of fund has been required to develop a synthetic antidote from poison itself.[35]

Palagarai chunnam is one of the traditional Siddha formulation which is indicated as a best drug for Female Infertility, Dysmenorrhea, Anemia, Dropsy in Siddha text[33]**.** As an initial step, in this present study, a part of standardization of this drug and its safety has been confirmed through necessary analysis and Acute & 28 days Repeated Oral Toxicity studies as per OECD guidelines.[36,37]

Standardization of the drugs means confirmation of its identity and determination of its quality and purity[38]**.** Standardization of the chunnam was confirmed by the methodology of the siddha text.

Physiochemical analysis of the palagarai chunnam **From Table 1,** shows that The Organoleptic characters shows that Palagarai chunnam is dull white in color and odorless powder form of drug with pH of 7.87%. reveals that, this is a slightly alkaline which is expected to have significant absorption in the in the stomach than in intestine. The loss on drying is less than 1% w/w, which explains its moisture content of test drug is very low. Loss on drying is loss of weight expressed as in percentage w/w resulting from water and volatile matter of any kind that can be driven off under specific condition[39]. It is easily soluble in water, alcohol and acetone and ether.

Ash values are helpful in determining the quality and purity of crude drugs, especially in powder form[40]**.** Total ash value is 97.01%, it implies that presence of inorganic constituents. Acid insoluble ash is 34.69%, and Water soluble ash is16.96%. This explains the purity of the test drug.

Qualitative analysis (Tables-3,4,5) of palagarai chunnam for Acid radicals, Basic radicals, and other constituents demonstrates the presence of Carbonate, Aluminum , Iron, zinc, calcium and alkaloids.

The **Inductively Coupled Plasma Optical Emission Spectrometry(ICP-OES)** analysis palagarai chunnam showed that the presence of physiologically important minerals like Iron, Calcium, Aluminum, Zinc and Magnesium. Heavy metals such as Mercury, Lead, Arsenic and Cadmium were found below the detectable limit.

The presence Zinc which is required for wound healing property[41] found in Palagarai may aid as topical application as an ointment for different kind of wounds also It is the second most abundant trace metal in humans after iron and it is the only metal which appears in all enzyme classes[42] and Zinc plays major role in the Reproductive organ developments, ripe for fertilization. Iron and calcium are the major element in human body manage the various biological activity.

The **Fourier Transform Infra Red Spectroscopy (FTIR)** analysis of Palagarai chunnam shows the presence of vibrational band observation around ~ 1420 to 1500 cm^{-1} and 860 to 875 cm^{-1} confirms is attributed to the presence of calcium carbonate [43].

The morphology of the Palagarai chunnam drug can be determined by **SEM.** The photographs shows that particles are spherical in shapes and sizes are in the range from **1 to 3 µm**. Thus, the size of the particle is capable to encourage the efficacy.

In Acute toxicity study (Table -7), carried out as per OECD guideline 423, there was no treatment-related death or signs of toxicity developed in albino rats at dosage levels of 300mg and 2000mg/kg body weight throughout the study period. Further, no gross pathological changes have been seen in the internal organs of both control and treated groups. This study reveals the Safety of the drug.

To confirm the safety of Palagarai chunnam, **28 days Repeated Oral Toxicity** Study was also carried out as per OECD test guideline 407. The animals were grouped and treated with different doses in study period as per guidelines. After 28 days blood collection done for animals of all groups. All the animals were euthanized for gross pathological examinations of all major internal organs. The blood samples were sent to a lab for hematological and biochemical analysis. The organs were weighed and preserved in 10% buffered formalin solution before sending for Histopathological study. All the reports were statistically evaluated.

Palagarai chunnam Substantial difference in Food and water intake the test group animals were observed when compared with control group during the study period(Table8,9 & Figure4,5) but they are within physiological limit, and this study exposes that it does not undesirably affect the basic metabolic processes of the experimental animals. In Hematological parameters, it had been observed that hemoglobin level was elevated after the administration of palagarai chunnam at the high dose level when it compared to control group animal(Table 12 & Figure 10). But the Hb level was gradually increased in test group compared to the control group but within normal range. In test groups there was significant changes present in Lipid profile, when compared with the control group. At the values were normal biological limits. (Table 13, Figure 12).

The Bio chemical parameters like Renal and hepatic parameters has significant changes but within normal range.(Table 14,15& Figure 13,14,15). The Histopathological study, organs such as brain, heart, kidney, liver, lungs, spleen and stomach were taken. In organs of Control and test group, no abnormality was detected. There’s no pathological changes occur in all group of animals during the study period.

7. SUMMARY

Palagarai chunnam is one of the traditional Siddha formulation which is indicated as a best drug for Female Infertility, Dysmenorrhea, Anemia, Dropsy in Siddha text Scientific validation of this formulation Palagarai chunnam have to be studied and the safety of the drug have to be ensured. The raw drug was procured from raw drug store. They were identified and authenticated by Zoological survey of India and Herbal plants were authenticated from Botanist, National Institute of Siddha. The Raw drugs were purified and the medicine was prepared as mentioned in the Siddha literature. On organoleptic examination, the finished product seems to be dull white in color and powder in nature.

The test drug was evaluated with Qualitative and Quantitative analysis to assess safety by acute and subacute toxicity studies. Standardization of the chunnam was confirmed by the methodology of the siddha text.

Total Ash value, Acid insoluble Ash value, Water, and Alcohol Soluble Extractive values reveal the purity of the test drug. Qualitative Analysis of test drug demonstrates the presence of Calcium, Iron, zinc, Aluminium and Alkaloids.

SEM results confirm the presence of micro-sized, spherical shaped particles ranging between 1 to 3µm, with a smooth surface in evenly distribution. **ICP-OES** analysis palagarai chunnam showed that the presence of physiologically important minerals like Iron, Calcium, Aluminum, Zinc and Magnesium.

The toxicological evaluations were conducted as per OECD guidelines 423 & 407 for safety evaluation of test drug. In acute toxicity study, no signs of toxicity and mortality were observed throughout the study period up to the dose of 2000mg/kg body weight but there is no abnormality was noted. In 28days Repeated dose Oral Toxicity Study, there was no significant changes in behavioral signs, food intake, water intake, Lipid Profile, Renal parameters hematological parameters and Hepatic parameters. In histological study of vital organs of Control group and high dose drug treated groups shows no abnormality. These findings were desire the safety of Palagarai chunnam used in Siddha system of medicines.

8. CONCLUSION

This study results that the Qualitative analysis of Palagarai chunnam reveals the Purity and Bioavailability of the drug Quantitative analysis expound the presence of essential trace elements in test drug which is import for various imperative biological activity for human body. In vivo toxicity studies indicate that there was no mortality and signs of toxicity observed for acute oral administration of test drug till the dose 2000 mg/kg b.wt in the recommended manner. In 28 days repeated oral toxicity study hematological and biochemical parameters are normal limits and no significant abnormality present in internal organs. Based on these results it can be conclude that, the dose level of kundri alavu **(130mg**) mentioned in **Siddha maruthuva nool thirattu - Anubhava Siddha Vaithiya Muraigal** is safety dose for human consumption.

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10. ANNEXURE

The following certificates are enclosed

1. Research Methodology and Biostatistics
2. Basic Research techniques and practices involved in Laboratory Animal care
3. IAEC Certificate for Acute and Sub Acute toxicity study
4. Authentication Certificate for herbal Plants
5. Authentication Certificate for marine drug (Palagarai)

NATIONAL INSTITUTE OF SIDDHA, CHENNAI – 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs taken up for Post Graduation Dissertation studies by **Dr.S.Balamurugan** M.D.(S), II year, Department of Nanju Nool, 2017, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology and Taxonomical methods as

Euphorbia ligularia Roxb. ex Buch.-Ham. (Euphorbiaceae), Whole plant

Citrus limon (Linn.) Osb. (Rutaceae), Fruit

DEPARTMENT OF MEDICINAL BOTANY ★ National Institute of Siddha ★ CHENNAI 600 047

Certificate No: NISMB3012017

Date: 12-06-17

Authorized Signatory

Dr. D. ARAVIND, M.D.(s),M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha
Chennai - 600 047, INDIA

CERTIFICATE

This is certify that the project title..... Pre-clinical Safety evaluation of the Herbo-Maine formulation 'Palsagarsichurnam" **.....**

Hasbeen approved by the IAEC. Approval No: NIS/IAEC#/08/2016

49-Rats (20M+29M)

Prof. Dr. V. Baramathi

Name of Chairman/~~Member Secretary~~ IAEC:

V. Baramathi 25/08/16

Signature with date

V.

Chairman/~~Member Secretary~~ of IAEC:

Prof. Dr. K. Nachimuthu.

Name of CPCSEA nominee:

25/8/2016

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

GOVERNMENT OF INDIA
Ministry of Environment, Forest and Climate Change
ZOOLOGICAL SURVEY OF INDIA

F.No 4-49/2015/Tech./ 229

29 March 2017

To
Dr.S.Balamurugan
Department of Nanju Nool
National Institute of Siddha
Chennai -47

Identification Report

The identity of the seven examples of shells submitted by you and here is the identification as follows

Phylum : Mollusca
Class : Gastropoda
Order : Littorimorpha
Family : Cypraeidae

1. ***Monetaria moneta*** **(Linnaeus, 1758)**

Material examined: 7 examples : **ZSI/MBRC/M. 2019.**

Coll: S.Balamurugan Dept.Nanju Nool, National Institute of Siddha, Chennai.

(Dr.Rajkumar Rajan)
Scientist-D &
Officer-In-Charge
प्रभारी अधिकारी
Officer-in-Charge
समुद्रीय जीव विग्नान क्षेत्रीय केंद्र
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NATIONAL INSTITUTE OF SIDDHA

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Tambaram Sanatorium, Chennai- 600 047

Workshop on

"BASIC RESEARCH TECHNIQUES AND PRACTICES INVOLVED IN LABORATORY ANIMAL CARE"

06 -10 February 2017

CERTIFICATE

This is to certify that Dr. S. Balamurugan has participated as Delegate/~~Resource~~ Person in the workshop on "Basic Research Techniques and Practices involved in Laboratory Animal Care" held on 06-10 February, 2017 at National Institute of Siddha, Chennai-47, Tamilnadu.

Dr. V. Suba
Organizing Secretary

Dr. P. Muthusamy
Veterinary Consultant

Prof. Dr. V. Banumathi
Director / Chairperson

The Tamil Nadu Dr.M.G.R.Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

This Certificate is awarded to Dr/~~Mr/Mrs~~.....S:...BALAMURUGAN.....

For participating as ~~Resource Person~~ / Delegate in the Twenty second Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

*For **AYUSH Post Graduates & Researchers***

Organized by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University From 06th to 10th June 2016.

Dr.N.KABILAN, M.D.(S)
PROF & HEAD
DEPT.OF SIDDHA

Prof.**Dr.S.PUSHKALA,** M.D.,
REGISTRAR (FAC)

Prof. **Dr.S.GEETHALAKSHMI,** M.D., Ph.D.,
VICE CHANCELLOR